EPIDEMIOLOGY AND EXOGENOUS FACTORS IN NOCTURNAL AIRFLOW LIMITATION IN CHILDREN

Gerda Rosman-Meijer

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VOORWOORD

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CHAPTER 1

INTRODUCTION

CHAPTER 1.1

EPIDEMIOLOGY AND THE CONCEPT OF UNDERLYING MECHANISMS OF NOCTURNAL ASTHMA

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Abstract

Nocturnal symptoms are common in asthma, even when patients are regularly seen at an outpatient clinic. Inflammation is generally accepted as a general feature of asthma and the severity of this basic inflammatory process can be increased by exogenous triggers such as exposure to allergens and non-allergic stimuli.
Superimposed endogenous circadian rhythms may play a more important and intricate role in the circadian modulation of the inflammatory process by changing the number of cells, their release of mediators and/or the susceptibility of airway smooth muscle and vasculature. For instance, an increase in vagal tone may induce nocturnal bronchoconstriction which is further enhanced by falling catecholamine levels. Together, the reduced nocturnal catecholamine levels and the diminished bronchodilating capacity of the NANC system and the low cortisol levels oppose possible protection against inflammatory processes leading to nocturnal airflow obstruction.

Introduction

Nocturnal symptoms of dyspnoea and wheezing are common in asthmatic patients and are thought to be related to the severity of the disease (1). Both in healthy subjects and asthmatic patients a circadian variation in airway diameter exists, with best lung function values during the day and worst values at night. In healthy subjects differences between daytime and nighttime values are small, in asthmatic patients they may be large with the consequence of nocturnal airflow limitation and symptoms such as cough, wheeze, dyspnoea and waking up.
Although nocturnal complaints of asthma have been recognized for a long time, little is known about its epidemiology. The mechanisms that contribute to the amplified 24 hours swings in pulmonary function are not yet fully clear but the concept is growing. In this article we will focus on both epidemiology and pathophysiology and try to come to a concept of mechanisms that play a role in nocturnal asthma.

Epidemiology

Throughout history the occurrence of nocturnal complaints of asthma have been reported (2).
In 1973 the first epidemiological study on the prevalence of nocturnal asthma appeared and was repeated in 1988. Turner-Warwick showed in 1988 that 39% of a non-hospital-based population in the United Kingdom woke up every night, 64% woke up at least 3 nights per week and 74% woke up at least 1 night per week (3).

These data were comparable to those of the study 15 yrs earlier, and the author concluded that not much had changed despite the introduction of inhaled corticosteroids.
In 1991 we studied the epidemiology of nocturnal complaints and early morning dyspnoea in 796 asthmatic children from our own outpatient clinic. The questionnaire referred to the last 3 weeks before a regular control visit. Forty seven percent reported nocturnal complaints or early morning dyspnoea. Only 6% reported to have complaints every night. In our population as well as in the study population from the United Kingdom about half of the patients used inhaled corticosteroids, drugs known to reduce the 24 hour amplitude of the lung function. The difference in results between the two studies may be explained by differences in age of the study populations and probably differences in frequency of medical control, since the population studied by Turner-Warwick was only defined as patients for whom an inhaled bronchodilator was prescribed by a general physician. Despite differences in outcome of the two studies, we have to conclude that nocturnal symptoms of asthma are still common in the nineties even in a regularly controlled asthmatic population.

Mechanisms

Both exogenous triggers, and probably more important, circadian variations in endogenous mechanisms, such as bronchial responsiveness, the autonomic central nervous system and cortisol secretion, modulate the inflammatory processes in the airways underlying the asthmatic expression. This may ultimately result in variation of the airway diameter over 24 hours.

BRONCHIAL RESPONSIVENESS

The increase in airflow obstruction during the night was thought to be a consequence of the increase in bronchial responsiveness that was observed at the same time (4). However, in asthmatic children we found that the circadian variation in bronchial responsiveness can be independent of the degree of airflow obstruction (5) as confirmed by Bonnet *et al.* (6). This indicates that an increase in bronchial responsiveness overnight in itself is not responsible for nocturnal airflow obstruction and that other factors are involved.

CORTISOL

Corticosteroids are well-known for their beneficial effect in restoring pulmonary function in severe asthma attacks with a latency of several hours. The circadian variation in serum cortisol shows trough levels at 01.00 h and peak levels about 08.00 h. Postma *et al.* (7) observed levels of serum cortisol in patients with chronic obstructive pulmonary disease (COPD) comparable to those in the matched healthy

controls. Together with the observation that infusion of cortisol did not prevent the nocturnal fall in pulmonary function in five out of six patients (8) has led to the believe that the fall in cortisol during the early night is not in itself responsible for the decrease in airway diameter at 04.00 h.

THE AUTONOMIC NERVOUS SYSTEM

The circadian variation in airway diameter is known to be under control of various components of the autonomic nervous system such as the parasympathetic system, ß-adrenergic sympathetic system and the non-adrenergic non-cholinergic (NANC) system.

The parasympathetic system

Increased parasympathetic tone causes a decrease in the airway diameter. In adult patients with asthma and in patients with COPD an increase in parasympathetic activity was observed during the night (9,10). We were unable to confirm this finding in children with nocturnal asthma (11). Moreover, after heart-lung transplantation, when vagal innervation is lost, a circadian variation in airway diameter is still present (12). These observations indicate that parasympathetic activity contributes, but does not fully explain nocturnal airflow obstruction.

The ß-adrenergic system

The ß-adrenergic system is of importance for airway dilatation and consists of circulating catecholamines and ß-receptors on cell membranes of inflammatory cells, cholinergic ganglia and airway smooth muscle. Circulating catecholamines such as adrenaline and noradrenaline show a circadian variation with lowest levels at 04.00 h coinciding with the nocturnal increase in airflow obstruction. $ß_2$-receptor density on peripheral blood cells is also lowest during the night. These observations suggest that the diminished bronchodilating capacity during the night is responsible for the nocturnal increase in airflow obstruction. However, correction of the nocturnal dip in serum adrenaline by infusion of adrenaline did not prevent the fall in pulmonary function (10). Moreover, we did not observe differences in adrenaline and noradrenaline urinary excretion between asthmatic children with and without increased nocturnal airflow obstruction and their healthy controls (13). This indicates that a fall in circulating catecholamines during the night does not have a direct action on bronchial smooth muscle tone. This fall will provide a smaller protective effect on for instance mast cells, thereby inducing histamine and other mediator release (13). Furthermore, increasing vagal tone can be opposed by inhibition of cholinergic neurotransmission at the level of parasympathetic ganglia and may permit an increase in microvascular leakage, ultimately leading to an increase in airflow obstruction.

The non-adrenergic non-cholinergic system

A circadian variation in NANC neurotransmission has been found as well: a decreased bronchodilator response upon stimulation with capsaicin was found at 04.00 h as compared to 16.00 h in healthy subjects and in asthmatics (14). This decreased bronchodilator response in the morning may result from central modulation of the stimulus or from inhibition of the efferent activity. The authors concluded that nocturnal airflow obstruction in asthmatic subjects may be partly caused by a decreased NANC bronchodilatation.

Concept of mechanisms in nocturnal asthma

It is generally accepted that a specific inflammatory process underlies the pathogenesis of asthma. That an inflammatory process may play a role in nocturnal asthma is supported by the observation that anti-inflammatory drugs such as inhaled corticosteroids reduce the overnight fall in pulmonary function (15).
Martin *et al.* (13) showed, at least in some, but certainly not in all patients with nocturnal asthma an increase in numbers of eosinophils and neutrophils in the nocturnal bronchoalveolar lavage (BAL) fluid as compared to daytime numbers. Jarjour *et al.* (16) did not observe day-night variations in inflammatory cells in the BAL fluid. Lungbiopsies will probably provide an answer whether an influx and activation of inflammatory cells in the lungs overnight are responsible for the fall in pulmonary function.
At this time we hypothesize the inflammatory process to be a general feature in asthma. The severity of this basic inflammatory process can be increased by exogenous triggers, such as exposure to allergens and non-allergic stimuli. Superimposed endogenous circadian rhythms may play a more important and intricate role in the circadian modulation of the inflammatory process by changing number of cells, their release of mediators and/or the susceptibility of airway smooth muscles and vasculature. Increased vagal tone may induce nocturnal bronchoconstriction. Falling catecholamine levels overnight may induce further decrease of the airway diameter. Together with the reduced nocturnal catecholamine levels, diminished bronchodilating capacity of the NANC system and low cortisol levels oppose possible protection against inflammatory processes, leading to nocturnal airflow obstruction.

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CHAPTER 1.2

AIMS OF THE THESIS

In this thesis several exogenous factors that may influence the severity of nocturnal airflow limitation in asthmatic children are investigated. Next to the influence of these exogenous factors we investigated the frequency of nocturnal symptoms on our pediatric asthma outpatient clinic.

In *Chapter 1.1* a concept of possible mechanisms and interactions between endogenous and exogenous factors that may underly nocturnal airflow limitation is discussed.

In *Chapter 2* we investigated the frequency of nocturnal symptoms such as coughing, wheezing, shortness of breath and dyspnea on awakening in the morning in our asthmatic outpatient clinic in children with asthma.

In *Chapter 3* a study is presented in which we investigated whether house dust mite exposure levels in houses of asthmatic children are higher than in houses of healthy controls.

In *Chapter 4* a study is presented in which we investigated the wheter exogenous factors such as environmental tobacco smoke, the presence of pets, and the levels of house dust mite in houses of asthmatic children with a mono-allergy to house dust mite contributed to an increased circadian peak expiratory flow amplitude.

In *Chapter 5* a study is presented in which we investigated whether the seasonal variations in house dust mite exposure contributed to an increase in circadian peak expiratory flow amplitude in asthmatic children with a mono-allergy to house dust mite.

In *Chapter 6* we discussed that mite-specific IgE could not be used as an alternative for house dust mite exposure in answer to a study in which the authors suggested that that this could be done.

In *Chapter 7* a study is presented in which we investigated in asthmatic children who were already treated with inhaled corticosteroids whether 16 weeks of treatment with the long acting ß-adrenergic drug salmeterol leads to a sustained bronchodilator effect and decreased bronchial responsiveness during the day and night. Furthermore, we assessed whether cessation of salmeterol after 4 months, when added to a regime with inhaled corticosteroids, leads to a rebound increase in bronchial responsiveness.

In *Chapter 8* a study is presented in which we investigated daytime and nighttime inflammatory parameters in healthy children and in asthmatic children treated with inhaled corticosteroids. Moreover, we assessed whether differences in inflammation

between healthy and asthmatic children are associated with lung function parameters and whether long-term treatment with salmeterol influenced inflammatory parameters.

CHAPTER 2

FREQUENCY OF NOCTURNAL SYMPTOMS IN ASTHMATIC CHILDREN ATTENDING A HOSPITAL OUTPATIENT CLINIC

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Abstract

Since nocturnal symptoms indicate more severe asthma we investigated the frequency in a hospital-based population of asthmatic children. Recognition of these symptoms offer the possibility to introduce appropriate treatment. We studied 796 consecutive children with asthma (mean age (SD) age 9 (4) yrs) attending a hospital clinic, to determine whether these nocturnal symptoms predicted that daytime activities would be affected, and also the patients' perception of disease severity. At the end of a regular outpatient clinic visit, the answers to seven different questions concerning nocturnal symptoms in the previous 3 weeks were recorded. The forced expiratory volume in one second (FEV_1) was ≥ 90% predicted in 98% of the population that was able to perform lung function measurements (72% of the total population). In 38% of the patients with nocturnal symptoms, these symptoms were reported spontaneously. When asked for, nocturnal symptoms were reported by 47% of the children; 6% every night and 34% at least once a week. Cough was the most frequently reported symptom (31%). Children with nocturnal symptoms had a lower FEV_1, scored their perception of asthma as more severe, and had their daytime activities affected more than those without nocturnal symptoms. Doctors should specifically ask about nocturnal symptoms, as not all patients with nocturnal symptoms report them spontaneously and they predict more severe disease.

Introduction

A circadian variation in peak flow rate exists both in healthy subjects and asthmatics, with best lung function values during the day and worst values during the night (1-3). The difference is due to a change in airway size at night (4). Nocturnal dyspnoea, cough, wheeze as well as dyspnoea on wakening in the morning are common symptoms in asthmatic patients (5). Dyspnoea on wakening in the morning is an indicator of the nocturnal fall in lung function at night. In children, it may cause patients and their parents to wake at night, and may lead to poorer educational and cognitive performance (6,7).

Nocturnal symptoms of asthma have been recognized for a long time (8). Since they indicates severe asthma, these symptoms would be expected to be common in a hospital-based populations of asthmatics. We investigated the frequency and types of nocturnal symptoms in children with stable asthma, who regularly visited our outpatient clinic, and also their effect on perception of asthma severity and daytime activity.

Patients and methods

Study Design
Children who were regular clinic attenders participated in our study between September 1990 and September 1991. All answered seven different questions concerning nocturnal symptoms in the previous 3 weeks. Questions were answered by the child or by the parents of younger children at the end of the outpatient visit after the usual history, physical examination and lung function tests had been completed.

Patients
All children were previously diagnosed as having asthma based on a history of recurrent episodes of wheeze, dyspnoea and coughing in response to allergens or non-allergic stimuli. History was taken according to the Dutch version of the standardized questionnaire of the British Medical Research Council (MRC European Coal and Steel Community (ECCS) questionnaire) (9).
Patients with viral infections or asthma exacerbations during the outpatient visit were excluded from the study.

Questionnaire
The questionnaire concerning nocturnal symptoms in the previous 3 weeks was administered by one of the 4 authors (GGM, JG, KK, WMCvA). Spontaneously reported nocturnal symptoms were noted. For this study, questions concerning nocturnal asthma were, deliberately, not asked by the physician before the questionnaire. All medication was recorded.
The following questions were asked:

1. Do you (does your child) wake up at night due to a) dyspnoea, b) cough, c) wheeze, d) do you (does your child) suffer from dyspnoea on wakening in the morning? Possible answers were yes or no? [Type of symptoms].
2. How often per week do you (does your child) suffer from symptoms mentioned under 1? [Frequency].
3. Do you consider the severity of your asthma (the asthma of your child) as mild, moderate, severe or very severe [Severity].
4. If there are nocturnal symptoms, do they generally influence activities during the next day?

The terms between square brackets are used to indicate the different questions in Tables 1 and 2.
The study protocol was approved by the University Hospital Ethical Committee.

Lung Function Measurements
In children of 6 yrs and older (n = 576), 72% of the population under study, forced expiratory volume in one second (FEV_1) was measured with a water-sealed

spirometer (Lode bv, Groningen, the Netherlands). The best of three FEV_1 efforts was used for statistical analysis. Normal values from Zapletal *et al.* were used (10).

Statistical Analysis
Statistical analysis was performed using SPSS/PC, version 4.0 (SPSS Inc., Chicago, USA). Values for age and FEV_1 (expressed as percentage of the predicted value [%pred]) had a normal distribution (Kolmogorov-Smirnov test), and are presented as means and standard deviations (SD). The group difference for FEV_1 has been analyzed with a Student's *t* test. Comparison between children with one or more affirmative answer(s) to the questions on nocturnal symptoms and those children who answered these questions negatively, have been analyzed by Chi-square test. For the relation of FEV_1 with the subjectively estimated severity grades, analysis of variance (ANOVA) was used. The analysis of the dependent variables for the estimation of subjective perception of the severity of the disease have been assessed with logistic regression and are presented as Odds ratio (OR) and 95% confidence interval (95% CI). A difference of 5% was considered as significant.

Results

Patients
Patients (n = 796, 512 boys (64%) and 284 girls (36%); mean (SD) age 9 (4) yrs) were labelled nocturnal asthma positive (NA+) when one or more of the questions on nocturnal symptoms were answered affirmatively, and nocturnal asthma negative (NA-) when these questions were answered negatively.

FEV_1
Ninety eight percent of the 576 children who performed lung function measurements had a $FEV_1 \geq 90$ % pred. Mean FEV_1 values were significantly lower ($p < 0.01$) in the NA+ group than in the NA- group (97.5 ± 6.5% and 98.8 ± 2.9%, respectively).

Type of Symptoms and Frequency
Almost half of the children (375 = 47%) had nocturnal symptoms. Cough was the most frequently reported symptom (31%), although it often occurred in combination with other symptoms. Dyspnoea at night (25%), dyspnoea on wakening in the morning (25%) and wheeze (18%) were less frequently reported. Nocturnal symptoms were present on every night in 6% of the children, and 34% reported having nocturnal symptoms at least once a week (Table 3). The NA+ group used significantly ($p < 0.01$) more maintenance medication (inhaled corticosteroids, cromolyn sodium, and ketotifen) than the NA- group (Table 4).

Subjective Perception of Severity of Asthma

Subjective perception of asthma was considered to be mild or moderate in 88% of the children, and severe or very severe by 11% (Table 1). Subjective perception of severity by patients in the NA+ group was significantly different from the NA- group ($p < 0.01$); more children in the NA+ group regarded their asthma as severe or very severe. Mean FEV_1 values did not vary between the four groups with different subjective perception of severity grades (mild, n = 317: 98.5 ± 2.9%; moderate, n = 212: 97.9 ± 6.9%; severe, n = 44: 97.7 ± 2.7%; very severe, n = 3: 99.4 ± 1.3%; ANOVA p = 0.47).

Daytime activities were influenced by nocturnal symptoms in 33% of the total population, this was 46% of the NA+ group and 20% of the NA- group ($p < 0.01$). The 20% of the NA- group were those who reported that before the 3 weeks of the questionnaire daytime activities were influenced by nocturnal symptoms.

Nocturnal symptoms were spontaneously reported in 25% of the total population, this was 38% of the NA+ group and 12% of the NA- group ($p < 0.01$). The 12% of the NA- group were those who spontaneously reported that their nocturnal symptoms that were apparent before the 3 weeks of the questionnaire had disappeared.

Table 2 shows the distribution of type and frequency of nocturnal symptoms by perceived severity of asthma in the population. The difference between mild *versus* moderate, severe and very severe was discriminated by nocturnal wheezing (OR: 2.45; 95% CI: 1.54 - 3.94), dyspnoea on wakening in the morning (OR: 1.90; 95% CI: 1.26 - 2.86) and the frequency of nocturnal symptoms (OR: 1.35; 95% CI: 1.20 - 1.52). Moderate *versus* severe and very severe disease were discriminated by dyspnoea at night (OR: 2.31; 95% CI: 1.36 - 3.94) and dyspnoea on wakening in the morning (OR: 1.90; 95% CI: 1.12 - 3.22).

TABLE 1 Subjective perception of severity of asthma by patients or their parents

	Total n = 796		NA+ n = 375			NA- n = 421		
	n	%	n	%	(% of total)	n	%	(% of total)
'Severity' *								
Mild	401	50	127	33.9	(16)	274	65.1	(34)
Moderate	304	38	176	46.9	(22)	128	30.4	(16)
Severe	79	10	62	16.5	(8)	17	4.0	(2)
Very severe	6	1	6	1.6	(1)	-	-	-
Unknown	6	1	4	1.1	(1)	2	0.5	(0)

Total: all subjects; NA+: subjects with nocturnal symptoms; NA-: subjects without nocturnal symptoms. *: Significant differences between NA+ and NA- group; $p < 0.01$.

TABLE 2 Subjective perception of severity of asthma by type and frequency of nocturnal symptoms

	Mild (n=401)	Moderate (n=304)	Severe (n=79)	Very severe (n=6)
Type of symptoms				
Dyspnoea at night	58	93	44	4
Cough	84	111	40	4
Wheeze	31	74	33	4
Dyspnoea on wakening in the morning	53	98	44	3
Frequency				
0 or < 1 /week	329	164	26	2
1x / week	27	31	12	-
2x / week	14	38	10	1
3x / week	10	26	8	1
4-6x / week	10	18	12	1
Every night	11	27	11	1

TABLE 3 Frequency of nocturnal symptoms in 796 children with stable asthma who were regular clinic attenders

Nocturnal symptoms	n	%
None	421	53
Less than once a week	102	13
Once a week	71	9
Twice a week	65	8
Three times a week	46	6
Four - six times a week	41	5
Every night	50	6

TABLE 4 Patient medication

	Total n = 796		NA+ n = 375		NA- n = 421	
	n	%	n	%	n	%
None						88
11 17 5	71	17				
Maintenance medication *						
None	123	16	39	10	84	21
Corticosteroids #	398	50	190	51	208	49
Cromolyn sodium #	190	24	100	27	90	21
Ketotifen	85	10	46	12	39	9
Bronchodilators						
Salbutamol #						
Regular	159	20	98	26	61	15
On demand	423	53	222	59	201	48
Ipratropium bromide #						
Regular	24	3	18	5	6	1
On demand	1	0	1	0	-	-
Theophylline	22	3	18	5	4	1

Total: all subjects; NA+: subjects with nocturnal symptoms; NA-: subjects without nocturnal symptoms. #: inhaled. *: NA+ group used more ($p < 0.01$) maintenance medication than the NA- group.

Discussion

Although nocturnal symptoms are considered to be a common phenomenon in asthmatic patients, nothing is known about their frequency in a hospital-based population. In a cross-sectional survey in our paediatric population, approximately half of the patients suffered from nocturnal symptoms of asthma during the 3 weeks before their regular outpatient clinic visit. Nevertheless, 89% of the patients regarded their asthma to be moderate or mild. Patients with nocturnal symptoms of asthma had a significantly lower FEV_1, reported often that their nocturnal symptoms influenced daytime activities, and assessed their asthma as severe significantly more often than those without nocturnal symptoms. Only 38% of the parents and patients with nocturnal symptoms reported it spontaneously. Results of the routine outpatient FEV_1 values were a poor predictor of nocturnal symptoms. Cough was the highest reported symptom.

Connolly (11) estimated that one third of adult patients with asthma attending a clinic suffered from nocturnal symptoms. In 1988 Turner-Warwick (5) showed that 39% of the patients in a non-hospital-based population woke every night, 64% woke at least 3 nights per week and 74% woke at least 1 night per week. These percentages were very close to those of a similar study by the same author fifteen years earlier. It was therefore concluded that the frequency of nocturnal symptoms of asthma had not diminished despite the introduction of newer drugs, such as inhaled corticosteroids.

Our study shows a lower frequency of nocturnal symptoms than reported in the United Kingdom (5), a difference which is likely to be due to selection of patients. We studied a hospital-based population of asthmatic children, and the use of medication was not an inclusion criteria. Turner-Warwick (5) studied patients of no specified age to whom a general physician had prescribed or represcribed an aerosol bronchodilator. Differences in asthma management may be another factor. Our asthmatic children visit our outpatient clinic at least once every 6 months, where the clinical history is recorded, and a physical examination and spirometry are performed.

Medication use was not different between our population and the population studied by Turner-Warwick (5). Forty eight percent of the latter study population also used inhaled corticosteroids, while 50% of our children used this type of medication. Despite the differences in outcome of the two studies, we conclude that nocturnal symptoms of asthma are still common, even an asthmatic population regularly attending an outpatient clinic.

Foo and Sly (12) investigated baseline pulmonary function with symptom scores and home monitoring of peak expiratory flow (PEF) variability in 100 clinically stable asthmatic children from their outpatient clinic. They found that one third had an abnormal FEV_1. We found that the majority (98%) of our investigated population had FEV_1 values within the normal range ($FEV_1 \geq 90\%$ pred). Nevertheless we observed a high frequency of nocturnal symptoms indicating that the disease is not stable.

Cough was the most frequently reported symptom. Falconer *et al.* (13) investigated the correlation between subjective reports of nocturnal symptoms and objective measurements of PEF recordings and voice activated tape recordings of coughing. They found a poor correlation between subjective and objective assessment of nocturnal symptoms. Their observations indicate that our results, based on a questionnaire, may underestimate the real frequency of nocturnal airway obstruction or cough. However, waking up will probably have a greater impact on daytime activities than the frequency of recorded coughing sounds.

Dyspnoea on wakening in the morning is a result of the nocturnal fall in lung function. In an earlier study, we observed that the 8:00 a.m. PEF value correlates well with the 4:00

a.m. value in a group of asthmatic children with increased airway obstruction overnight (3).

In the current study we labelled 375 (47%) of the population as having nocturnal symptoms. If we had not asked about dyspnoea on wakening in the morning, 314 (39%) would have been labelled as having nocturnal symptoms. We would than have missed about 8% who slept through their nocturnal airway obstruction.

A surprising observation in our study was that nocturnal symptoms were only spontaneously reported in 38% of those experiencing them. It may be assumed that children and/or parents are accustomed to nocturnal symptoms and consider them as a normal feature. As nocturnal asthma is a sign of instability of asthma and associated with a higher rate of exacerbations (4), our findings have important implications for clinical practice. As stressed earlier by Henry *et al.* (14), physicians should specifically ask for nocturnal symptoms, since these are often not spontaneously reported. Since asthma has different presentations and symptoms may differ with age group, we emphasize that all four symptoms should be asked about. Although we did observe significantly lower FEV_1 values in the group with nocturnal symptoms than in the group without nocturnal symptoms, the observed difference is small and mean FEV_1 values of both groups were in the normal range.

Our study indicates that routine outpatient clinic spirometry is a poor predictor for nocturnal symptoms. A more objective assessment of nocturnal airway obstruction is the measurement of PEF values on wakening in the morning (3,14). This may help both parents and physicians to assess the severity of nocturnal airway changes. Moreover, it provides an indication of the severity of the disease and offers the possibility to introduce appropriate treatment.

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CHAPTER 3

HOUSE DUST MITE EXPOSURE IN ASTHMATIC AND HEALTHY CHILDREN: THE DIFFERENCE IS CARPETING

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Abstract

Aim - To determine whether house dust mite (HDM) exposure in living rooms and bedrooms is higher in asthmatic children than in those of age and sex matched - healthy children, living in the same area. *Methods* - Types of floor-coverings were recorded and dust samples were collected by vacuum cleaning the total area of living rooms and bedrooms; *Der* p I and *Der* p II per gram of fine dust concentrations were assessed. Twenty-five asthmatic children (RAST HDM ≥ class 3, age 6-12 yrs) and 25 healthy children participated in the study. *Results* - The frequency of cleaning and prevalence of smooth floor-coverings in bedrooms of asthmatic children were significantly higher. There were no differences in living rooms in this respect. The amount of fine dust/m^2 floor space was significantly lower in bedrooms of asthmatic children. Concentrations of HDM were low and no differences in *Der* p I and *Der* p II levels were observed between the two groups (asthmatic children: *Der* p I living room: 1.1 (0.04 - 59.4 µg/g), bedroom: 0.5 (below detection - 19.3 µg/g); non-asthmatic children: *Der* p I living room: 1.4 (below detection - 27.5 µg/g), bedroom: 0.9 (below detection - 68.8 µg/g). Smooth floor-coverings contained significantly less fine dust, *Der* p I, and *Der* p II than carpeted floors. *Conclusions* - Low HDM concentrations are a general finding in Dutch dwellings in the present generation of children. We observed a higher cleaning frequency, and more smooth floor-coverings in bedrooms of asthmatic children than of healthy children, yet HDM concentrations were not significantly different. The latter can be explained by the observation that only 40% of the asthmatic children had smooth floor-coverings in their bedrooms. Smooth floor-coverings contain less fine dust and lower levels of *Der* p I and *Der* p II than carpeted floors.

Introduction

House dust mites (HDM) are the most important allergens for inducing asthmatic symptoms in allergic adults and children (1-6). Earlier studies have shown that exposure to allergens in newly diagnosed asthmatic adults is higher than in randomly selected controls (7,8). Recently Sporik *et al.* (5) suggested that, in addition to genetic factors, exposure to HDM allergens in early childhood plays an important role in the subsequent development of asthma. Therefore, reduction of environmental HDM concentrations appears to be of clinical and prognostic relevance in the management of allergic asthma in children.

In a recent study we have found low concentrations of *Der* p I and *Der* p II in floor dust of living rooms and bedrooms of asthmatic children (9). These HDM levels were remarkably lower than previously reported in studies from other countries with a similar climate (5,8,10). The lower concentrations of *Der* p I and

Der p II in our study may have been the result of either advice given in former years to reduce allergen exposure or a general lower concentration of HDM in houses of this generation of children. We therefore investigated whether we could find a difference in HDM concentrations in living rooms and bedrooms between allergic asthmatic children and age and sex matched healthy children. We compared types of carpeting, cleaning frequencies, age of the houses, humidity, and amounts of fine dust and HDM concentrations in floor dust.

Patients and Methods

We studied 25 allergic asthmatic and 25 healthy children, all attending primary school. The 25 consecutively selected asthmatic children visited the outpatient clinic for pediatric pulmonary diseases at our University Hospital. Routine standardised information with regard to reduction of environmental allergens was given previously by pediatricians and specially trained nurses at our outpatient clinic. These advices were given during the first visit and focused on HDM reduction in bedrooms.

Age and gender were simular between patients and controls (age: asthmatic children 9.1 ± 1.4 yrs, healthy controls 9.2 ± 2.1 yrs; gender (male/female): asthmatic children 14/11, healthy controls 15/10). Children with asthma were allergic to HDM, defined as a RAST ≥ class 3 (Pharmacia Diagnostics, Uppsula, Sweden) and had increased airway responsiveness, defined as a histamine provocation concentration (PC_{20} histamine) less than or equal to 16 mg/ml (2 min inhalation) (11). No acaricides, matress coverings or dehumidifiers were used in the investigated houses. All patients used inhaled corticosteroids, twice daily 200 or 400 µg, and short-acting ß$_2$-adrenergic drugs when needed. Short-acting ß$_2$-adrenergic drugs were withheld 8 h before the lung function measurements. A healthy classmate living in the same area of the city, was asked to participate in the study as a control. Controls never had respiratory complaints such as wheezing, dyspnea, or cough and were never diagnosed as allergic and/or asthmatic. In addition their first-degree relatives did not suffer from either asthma or any other allergic disease. All healthy children had normal total IgE values (12) and normal spirometric lung function values. Two children in the control group had increased RAST values (HDM 67, grass 8.5, dog 0.7 PRU/ml and grass 1.3, dog 16.6 PRU/ml) but were asymptomatic, and therefore included in the study. Analysis with and without these two controls showed no differences. Four children (4/25 = 16%) refused venous puncture and spirometry.

The study was approved by the Medical Ethics Committee of our hospital.

Study Design

The homes of the children were visited between March to May in the same year.

Measurements in the houses of patients and their controls were performed on the same day.

Methods

The way of carpeting, frequency of cleaning of living rooms and bedrooms, and the age of the houses were recorded on a checklist. Smooth floor-coverings were defined as hard floors that could be cleaned wet, while carpeted floor-coverings were defined as wall-to-wall carpeted floor-coverings either from synthetic, wool, or cotton material.

All floor dust samples were obtained by the same technician, using a vacuum cleaner (Phillips type T580, 1100 W). For every location a separate double-walled disposable paper bag was used (13). Dust was collected from the total area of the location in order to obtain a representative sample, vaccuum cleaning time per square meter was according the WHO International standards (14,15). The total amount of fine dust of each sample was measured after filtering with a 355-µm aperture sieve. Each sample was analyzed for the amount of HDM, and the concentration expressed in µg/g of fine dust according to the WHO International standards (14,15). The HDM allergens *Der* p I and *Der* p II were analyzed by sandwich immunoassays using monoclonal antibodies (16). A reference HDM extract was used, which was calibrated for the content of *Der* p I and *Der* p II by the Allergy Division of the Dutch Central Laboratory of the Blood Transfusion Service (Amsterdam), in serial dilutions for calculation of the allergen content of the HDM samples. The controlled samples in this reference laboratory resulted in comparable HDM concentrations. Air temperature and relative humidity were measured to obtain absolute humidity values (gram water vapor per kilogram of dry air) in each location.

Pulmonary Function Measurements

Spirometry (inspiratory slow vital capacity (IVC) and forced expiratory value in one second (FEV_1)) was performed with a water sealed spirometer (Lode BV, Groningen, The Netherlands). Peak expiratory flow (PEF) measurements were performed with a Wright peak flow meter. The best of three efforts was used for statistical analysis. Normal values from Zapletal *et al.* were used (17).

Statistical Analysis

Statistical analysis was performed by SPSS/PC, version 4.0 (SPSS Inc, Chicago, IL, USA). Variables with a Gaussian distribution are presented as means ± SD. Variables with a non-Gaussian distribution (HDM allergen concentrations) are presented as medians with minimum and maximum values. Differences were calculated by paired *t* tests and by nonparametric tests (Mann-Whitney *U*) as appropriate. Differences in discontinuous variables were calculated by Chi-squared tests. Correlations were calculated by Spearman's rank correlation coefficient. A p

value of < 0.05 was accepted as significant. When the amount of fine dust was less than 0.1 g per location, we used the 0.1 g values for calculations. When the allergen load was below detection concentration, we used the detection concentration (0.01 µg/g for *Der* p I and *Der* p II) for calculations. HDM values below detection concentration were not overestimated due to nonparametric testing.

Results

Total IgE, specific IgE to HDM and total number of eosinophils were significantly higher ($p < 0.05$) in the asthmatic children compared to the healthy children (total IgE: 359 (78 - 7699 IU/ml) versus 52 (4 - 674 IU/ml); specific IgE to HDM: 17.3 (2.1 - 180 PRU/ml) versus 0 (0 - 67.0 PRU/ml); eosinophils: $5.0 \pm 3.4\ 10^8$/l versus $2.9 \pm 2.0\ 10^8$/l). Lung function values were similar between the two groups.
Cleaning frequency of the living rooms were similar (asthmatic children: 3.5 (1 - 7) times per week, healthy children 2.5 (1 - 7) times per week). Cleaning frequency of the bedrooms was significantly higher in asthmatic children compared to healthy children (2.0 (1 - 7) times per week versus 1.0 (1 - 7) times per week, $p < 0.05$). The ages of the houses were similar (asthmatic children 20 (3 - 100 yrs), healthy children 20 (1 - 141 yrs). No significant correlation was found between cleaning frequency or age of the buildings on one hand and the amount of fine dust or allergen concentrations of both locations of the asthmatic and healthy children on the other hand.
We observed comparable types of floor-coverings in living rooms of both groups of children (smooth floor-coverings in asthmatic group: 9/25=36%; in the healthy group 8/25=32%), but significantly more smooth floor-coverings in the bedrooms of the asthmatic children (10/25 = 40%) than in the bedrooms of the healthy children (3/25 = 12%). In 2 bedrooms (1 asthmatic and 1 healthy child) we collected less than 0.1 g fine dust. These samples were not analysed for HDM.
The amount of fine dust/m^2 floor space in the living rooms of the asthmatic group was significantly higher ($p < 0.05$) than in the bedrooms. We did not observe this difference in the group of healthy children (Table 1). In the bedrooms of the asthmatic children significantly less ($p < 0.05$) fine dust was found on smooth floor-coverings than on carpeted floor-coverings, this difference being almost significant in the control group ($p = 0.09$). Smooth floor-coverings (measured for all children) contained less dust per squaremeter floor space than carpeted floor-coverings in general, in the separate locations, and in both study groups (Table 2).
The *Der* p I and *Der* p II concentrations in floor dust (Figure 1) were similar in living rooms and bedrooms, and no significant differences were found between the locations of the asthmatic and healthy children. In all locations some samples contained *Der* p I concentrations above international safety standards (14,15): in

the asthmatic group: 6 above 2 µg and 6 above 10 µg *Der* p I /g of fine dust; in the control group 7 above 2 µg and 6 above 10 µg *Der* p I /g of fine dust.
Table 2 shows the relationship of type of floor-covering to HDM concentrations per location expressed in µg/g of fine dust for *Der* p I and *Der* p II. Smooth floor-coverings contained significantly less *Der* p I than carpeted floor-coverings in general (measurements of all children), in the different locations and in both study groups (Table 2). The same was true for *Der* p II in the living rooms of both groups and in the bedrooms of the healthy children, as well as for the levels of *Der* p II on smooth and carpeted floors in the bedrooms, and in all combined locations of the asthmatic children.
When *Der* p I and *Der* p II are expressed in µg/m^2 floor space, all comparisons in Table 2 showed significantly less allergen in favour of smooth floors ($p < 0.01$).
No significant differences in humidity were found between living rooms and bedrooms of both groups of children. Values in the living rooms were 7.2 ± 0.9 g/kg and 7.7 ± 0.9 g/kg and in the bedrooms 6.2 ± 0.6 g/kg and 6.5 ± 1.1 g/kg, for the asthmatic and healthy children respectively. Absolute humidity in the bedrooms was significantly lower than in the living rooms in both groups. We did not find any significant correlation between absolute humidity and HDM allergen concentrations.

TABLE 1 Amount of fine dust and type of floor-covering in living rooms and bedrooms

	AAC		NAC	
Living room				
All floor-coverings	0.15	(0.01 - 1.81)*	0.11	(0.02- 0.89
Smooth	0.07	(0.01 - 0.27)	0.07	(0.03- 0.29)
Carpet	0.08	(0.03 - 1.81)	0.16	(0.02- 0.89)
Bedroom				
All floor-coverings	0.03	(0.01- 1.13)[1]	0.12	(0.01- 1.93)
Smooth	0.02	(0.01- 0.19)**	0.05	(0.02- 0.12)[2]
Carpet	0.14	(0.01- 1.13)	0.13	(0.01- 1.93)

AAC: allergic asthmatic children, NAC: non-asthmatic children. Values are expressed as median (minimum - maximun) value of gram fine dust/m^2 floor space in general or on type of floor-covering, significant differences by Mann-Whitney *U* test.

*: $p < 0.05$ between living room and bedroom in AAC; **: $p < 0.05$ between types of carpeting in AAC; 1: $p = 0.08$ between AAC and NAC for all floor-coverings; 2: $p = 0.09$ between types of carpeting in bedroom NAC.

TABLE 2 Amount of fine dust and house dust mite exposure in the locations by type of floor-covering

	'smooth'	'carpeted'
All locations	(n = 30)	(n = 70)
Dust, g/m^2	0.04 (0.01 - 0.29)	0.14 (0.01 - 1.93)*
Der p I	0.4 (n.d. - 33.0)	1.5 (n.d. - 68.8)*
Der p II	0.09 (n.d. - 13.3)	0.9 (n.d. - 59.7)*
All living rooms	(n = 17)	(n = 33)
Dust, g/m^2	0.07 (0.01 - 0.29)	0.16 (0.02 - 1.81)*
Der p I	0.3 (n.d. - 33.0)	1.6 (0.03 - 59.4)*
Der p II	0.09 (n.d. - 13.3)	0.6 (n.d. - 14.7)*
All bedrooms	(n = 13)	(n = 37)
Dust, g/m^2	0.02 (0.01 - 0.19)	0.13 (0.01 - 1.93)*
Der p I	0.4 (n.d. - 13.5)	1.1 (n.d. - 68.8)*
Der p II	0.09 (n.d. - 9.4)	1.0 (n.d. - 59.7)1
All AAC locations	(n = 19)	(n = 31)
Dust, g/m^2	0.03 (0.01 - 0.27)	0.14 (0.01 - 1.81)*
Der p I	0.4 (n.d. - 33.0)	1.1 (0.06 - 59.4)*
Der p II	0.10 (n.d. - 13.3)	0.5 (n.d. - 14.7)
All NAC locations	(n = 11)	(n = 39)
Dust, g/m^2	0.06 (0.17 - 0.29)	0.16 (0.01 - 1.93)*
Der p I	0.3 (n.d. - 1.7)	1.7 (n.d. - 68.8)*
Der p II	0.08 (0.01 - 4.1)	1.0 (n.d. - 59.7)*

Values are expressed as median (minimum - maximum) value, significant differences by Mann-Whitney *U* test. AAC: allergic asthmatic children, NAC: non-asthmatic children. *Der* p I and *Der* p II are expressed as µg/g fine dust. *: $p < 0.05$ between carpeting. 1: $p = 0.06$ between carpeting.

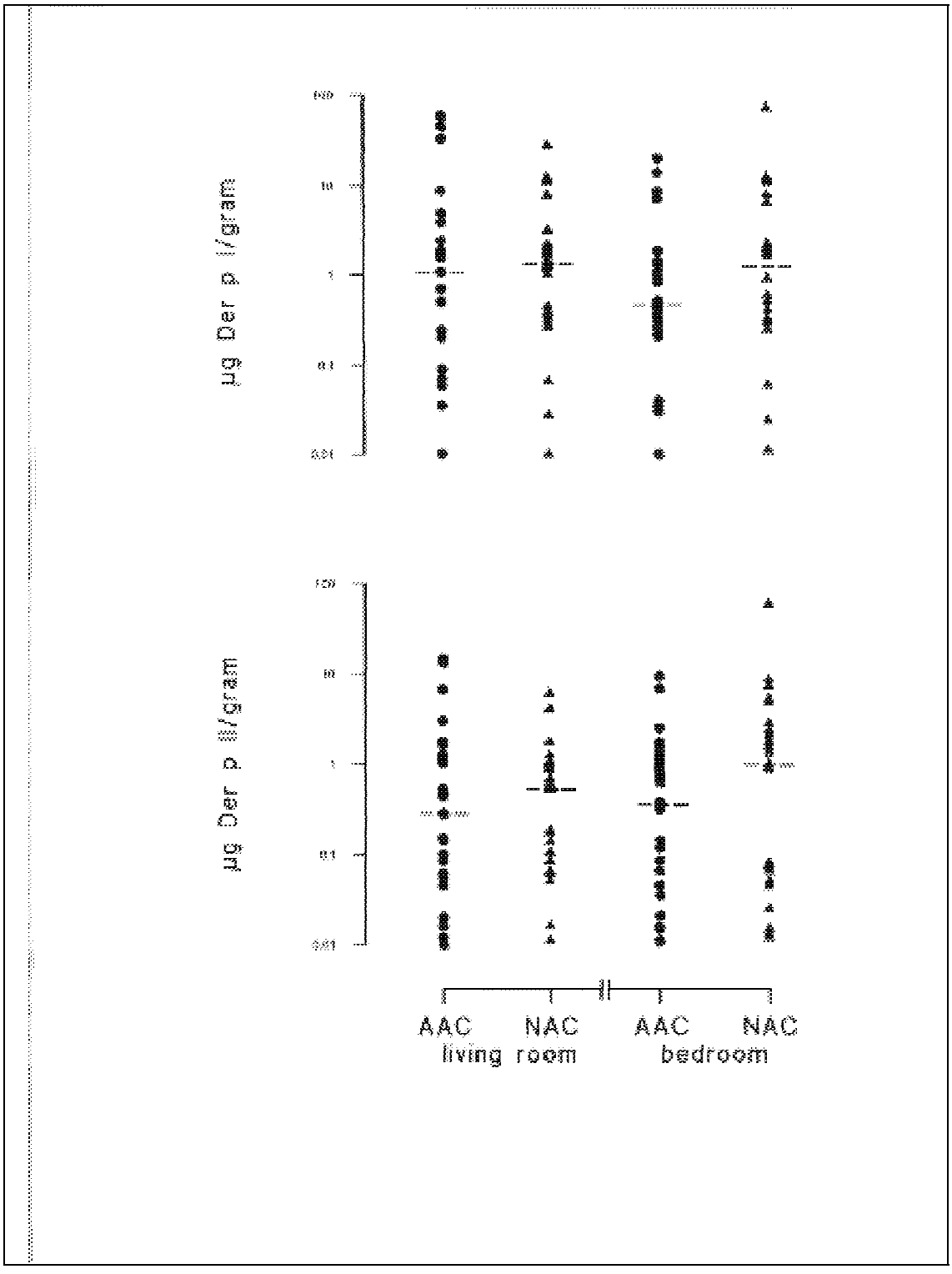

Figure 1 *Der* p I per gram of fine dust (µg/g) (*upper panel*), and *Der* p II per gram of fine dust (µg/g) (*lower panel*) in living rooms and bedrooms. Circles: values of the allergic asthmatic children (AAC), triangles values of the non-asthmatic children (NAC). --- : median value.

Discussion

We did not observe significant differences in *Der* p I and *Der* p II exposure between asthmatic and healthy children. As expected, smooth floors contained significantly lower *Der* p I and *Der* p II concentrations than carpeted floors.

The house dust mite (HDM) exposure levels were low as compared to those reported from other countries with a similar climate (5,8,10), confirming our previous findings (9). In that report, we hypothesised that the observed lower exposure concentrations were the result of previously given routine advice with regard to preventive measures to reduce HDM exposure. The results of this study reject this hypothesis.

Bedrooms of the asthmatics had significantly more frequent smooth floor-coverings than the control group and therefore contained less dust. When all smooth floor-coverings were compared with all carpeted floors we indeed observed significantly lower HDM concentrations on smooth floor-coverings. Although we observed that bedrooms of asthmatic children were more frequently cleaned, we could not find a correlation between cleaning frequency and the amount of fine dust or allergen concentrations.

High concentrations of HDM may be an important risk factor for both development and the severity of asthmatic symptoms in sensitized children (5,18). Our observation indicates that parents, probably as a result of former advice, focus on adjusting the type of floor-covering in the bedrooms of their children. This is in line with findings in Denmark (19), where houses of non-allergic individuals contained significantly higher amounts of cat allergen than houses from allergic individuals.

Our study shows that smooth floor-coverings are associated with less dust and lower HDM concentrations. It is known that HDM flourish at higher temperature and humidity, suggesting that reduction of humidity is important in the battle against HDM. We observed no differences in absolute humidity between the houses of the two study groups, and especially not in the bedrooms. This does not imply that humidity is not important. Korsgaard (7) found a higher number of HDM and a higher absolute humidity in his asthmatic group than in the control group. Absolute humidity values in his and our study are comparable. Moreover, we could not confirm the observed correlation between humidity and allergen concentrations. A difference between his and our study is that we only measured humidity at one point of time. Furthermore, measurements in the Danish study were performed in November and December, while our measurements took place from March to May. It may well be that humidity differences may exist at other times of the year.

We did not observe significant differences in *Der* p I and *Der* p II concentrations between asthmatic children and their controls in bedrooms and living rooms. According to the international standards for preventive measures to reduce HDM exposure, a concentration of 2 µg *Der* p I /g of fine dust should be a risk factor for sensitisation, and a concentration of 10 µg *Der* p I /g of fine dust a major risk factor for acute asthma in mite allergic individuals. Although the median values of *Der* p I in our study were rather low, in both types of locations some sample concentrations (14/100 = 14%) were above 10 µg *Der* p I /g of fine dust. Seventy-six percent of the allergic asthmatic children with maintenance medication of inhaled corticosteroids, were exposed to lower concentrations of *Der* p I than 2 µg/g of fine dust in living room and/or bedroom. This indicates that lower concentrations of *Der* p I are also of importance to the clinical expression of the disease (20), and that the international recommendations should be revised. The low HDM concentrations from our pediatric population are in accordance with observations in houses of adults from our region (16), and a recent publication on HDM concentrations from more southern regions in the Netherlands (18). Despite the observed lower HDM concentrations, all Dutch studies report a large individual variation. It seems, therefore, worthwhile to verify individual HDM concentrations in these locations and relate them to allergen reduction measures.

We conclude that the observed low HDM concentrations in Dutch dwellings are a general finding in this generation of children. We have observed a higher cleaning frequency, and a higher prevalence of smooth floor-coverings in bedrooms of asthmatic children than of healthy children, yet HDM concentrations were not significantly different. The latter can be explained by the observation that only 40% of the asthmatic children had smooth floor-coverings in their bedrooms. Finally we confirm previous data in that smooth floor-coverings contain less fine dust and lower concentrations of *Der* p I and *Der* p II.

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CHAPTER 4

EXOGENOUS STIMULI AND CIRCADIAN PEAK EXPIRATORY FLOW VARIATION IN ALLERGIC ASTHMATIC CHILDREN

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Abstract

The influence of exogenous factors in the home on the circadian variation of airway obstruction has not been fully assessed in children with asthma. The aim of the study was to investigate the contribution of exogenous stimuli to the degree of peak expiratory flow (PEF) variability during 24 h. Fifty-five children (33 boys and 22 girls; mean age 9.3 ± 1.7 yrs) with symptoms of asthma, increased bronchial responsiveness and a solitary allergy to house dust mite (HDM) participated. Their asthma symptoms were well-controlled for at least 4 mo with daily inhaled corticosteroids (ICS) and ß$_2$-adrenergic drugs if needed. Symptoms, peripheral blood eosinophils, total IgE, and specific IgE to HDM were assessed. Spirometry and PC_{20} histamine were performed. PEF amplitudes during 24 h (highest minus lowest as a percentage of day's mean value) were obtained at home during and 6 d after withdrawal of ICS. Dust samples were collected from the total area of the living rooms, bedrooms, mattresses (n = 25), and classrooms to obtain the HDM allergen (HDMA) exposure to *Der* p I and *Der* p II. Family smoking habits, presence of pets, and types of floor-covering were recorded on a checklist. Mean PEF amplitude did not increase after withdrawal of ICS, but absolute PEF values were significantly lower ($p = 0.05$) at midnight and 4:00 a.m. Twenty-six children (47%) were exposed to environmental tobacco smoke (ETS), 23 (42%) kept pets. Mattresses contained significantly higher amounts of HDMA compared with other locations. PEF amplitude after withdrawal of ICS was significantly higher in children exposed to ETS, a pet, or a high HDMA level in their mattress than in children who were not exposed (ETS: 29.7% [3.9-56.6] versus 19.4% [0.0-56.6], $p < 0.05$; pets: 31.4% [9.7-52.5] versus 21.9% [0.0-56.6] $p < 0.05$; high HDMA level in the mattress 35.5% [10.2-56.6] versus 21.4% [3.9-56.6] $p < 0.05$). These factors combined with age, PC_{20} histamine and its interaction with ETS, especially in mild to moderate asthma, explained 48.4% of the variance of the PEF amplitude after withdrawal of ICS. Exogenous stimuli such as exposure to ETS, pets, and high HDMA levels in mattresses contribute to an increased circadian PEF amplitude after withdrawal of ICS and therefore to nocturnal worsening of asthma in HDM-allergic asthmatic children. Moreover, ETS exposure seems especially to worsen PEF variability in children with mild to moderately severe bronchial responsiveness.

Introduction

Nocturnal airflow limitation is a common feature in children with asthma and its underlying pathophysiology has only partially been solved. Several endogenous factors such as circulating adrenaline and vagal activity (1,2), and exogenous factors such as exposure to tobacco smoke and to allergens may contribute to the

severity of asthmatic symptoms in children during the night. Allergen exposure seems especially important, because it has been shown that allergen provocation may increase circadian peak expiratory flow (PEF) variation for weeks on end (3). In an earlier study, we have observed a worsening of nocturnal PEF values after withdrawal of anti-inflammatory maintenance medication at home in children with allergic asthma (4). When these same children were admitted to a hospital and their medication was subsequently withdrawn, the opposite happened, i.e., nocturnal lung function values improved. This suggested that environmental stimuli (at home) contributed to the increased circadian swings in airway diameter after withdrawal of medication. Next to allergens, other factors such as environmental tobacco smoke (ETS) are known to contribute to increased airway obstruction (5,6). Therefore, the contribution of exogenous stimuli in the home to an increased circadian PEF amplitude after withdrawal of anti-inflammatory medication was investigated. We assessed associations of PEF amplitude with exposure to ETS, pets, and house dust mites (HDM) in a group of asthmatic children with a solitary allergy to HDM.

Patients and methods

Patients

Fifty-five asthmatic children (33 boys, 22 girls; mean age, 9.3 ± 1.7 yrs) visiting primary school were included. All were characterized by symptoms of asthma, an increased total IgE, and specific IgE to HDM (RAST class ≥ 3; Pharmacia Diagnostics, Uppsala, Sweden). None had increased specific IgE to dog, cat, tree, grass, or milk (RAST class = 0; Pharmacia Diagnostics) in order to exclude interference with other allergies and to obtain a more homogeneous population. All children had a forced expiratory volume in one second (FEV_1) as percentage of the predicted value ≥ 70% and increased bronchial responsiveness (histamine provocation concentration ≤ 8 mg/ml causing a fall of 20% or more in FEV_1 from baseline value). Symptoms were well-controlled with maintenance inhaled corticosteroids (ICS) for at least 4 mo before the study, twice daily 200 µg or 400 µg, and $ß_2$-adrenergic drugs when needed. Routine standardized information with regard to reduction of environmental allergens was given previously by the pediatrician and/or specially trained nurse at our outpatient clinic. Acaricides, mattress encasings, and dehumidifiers were not used in the investigated houses. Informed consent from all children and their parents was obtained, and the study was approved by the Medical Ethics Committee of our hospital.

Study Design

Daytime and nighttime symptoms were recorded during the 3 wk before withdrawal of ICS. At the end of the 3-wk period, a home and school visit was

made to collect house dust from living rooms, bedrooms, mattresses, and classrooms. Mattress samples were only collected from 25 of the 55 included children. Family smoking habits, presence of pets in the household, and type of floor-coverings were recorded on a checklist. Temperature and relative humidity were measured in each location to obtain absolute humidity (gram water vapor per kilogram of dry air).
At the outpatient clinic, a blood sample was drawn to determine eosinophil count, total IgE, and specific IgE to HDM; spirometry and a histamine inhalation challenge test were performed during treatment with ICS.
The circadian PEF amplitude (highest minus lowest PEF value expressed as percentage of the day's mean value) was obtained at home on two days: one day during ICS and on the sixth day after withdrawal of ICS. All measurements were performed in three consecutive years, from August to November.

Clinical Characteristics of the Subjects
SYMPTOMS: Symptoms of cough, wheeze, dyspnea, and phlegm production during the day and at night were recorded in a diary on a 4-points scale (0: no symptoms, 1: mild, 2: moderate, 3: severe) (7). Daily symptoms were recorded and added up during the 3-wk period to obtain total symptoms during daytime and nighttime during ICS. Symptoms were also recorded during daytime and nighttime on the sixth day after withdrawal of ICS.

LUNG FUNCTION AND PROVOCATION TESTING: PEF measurements were performed at home, every 4 h during 24 h, in an upright position with a mini-Wright peak flow meter. The best of three efforts was used for statistical analysis. FEV_1 and inspiratory vital capacity (IVC) were measured with a water-sealed spirometer (Lode BV, Groningen, the Netherlands). At least three reproducible values (i.e. < 5 % difference among the recordings) were obtained; the highest was used in the analysis. Airway histamine challenge tests were performed during ICS with a gauged DeVilbiss 646 nebulizer (DeVilbiss, Somerset, MA, USA), with an output of 0.13 ml/min according to the modification method of Cockcroft and colleagues (8). A 0.9% phosphate-buffered saline solution and doubling histamine concentrations from 0.03 to 16 mg/ml were inhaled for 2 min during tidal breathing, with the nose clipped, at 5-min intervals, until FEV_1 had fallen by 20% from the initial value. The exact provocation concentration of histamine that induced a 20% fall in FEV_1 (PC_{20}) was assessed by a log-dose response curve. $ß_2$-Adrenergic drugs were withheld 8 h before the measurements.

LABORATORY INVESTIGATIONS: Total IgE and specific IgE were quantified using an enzyme immunoassay procedure (Pharmacia Diagnostics), and expressed in international units (IU) per milliliter, and Phadebas RAST units (PRU) per milliliter, respectively.

Exogenous Stimuli

All dust samples were obtained by the same technician, using a vacuum cleaner (Phillips type T580, 1100 W). For every location, we used a different double-walled disposable paper bag (9), and a special vacuum cleaner filter for the mattresses (ALK filter device; surface, 38 cm^2, pore size, 6 µm; ALK allergolic lab, Hørsholm, Denmark). Dust was collected from the total area of the location in order to obtain a representative sample. The total amount of fine dust of each sample was measured after filtering with a 355-µm aperture sieve. Each sample was analyzed for the amount of HDM allergen (HDMA) (*Der* p I and *Der* p II) per gram of fine dust according to the WHO International standards (10,11). HDMA were analyzed by sandwich immunoassays using monoclonal antibodies (12).

Smooth (hard) floor-coverings, with or without a small carpet, were defined as floor-coverings that could be cleaned wet, while carpeted floor-coverings were defined as wall-to-wall carpeted floor-coverings either from wool, cotton, or synthetic material.

Statistical Analysis

FEV_1 values were expressed as percentage of the predicted value (% pred) (13). PC_{20} values were used after logarithmic transformation (base 2), since this reflects doubling doses and normalized the distribution. Total eosinophils, total IgE, and specific IgE HDM were logarithmically transformed (base 10) to normalize the distribution. Skewedness of distribution was assessed with a Kolmogorov-Smirnov test. If a p value < 0.05 was obtained, nonparametric techniques (Spearman's rho for correlation, Mann-Whitney *U* test to compare group means) were applied to analyze the data, values being expressed as median (minimum to maximum). Otherwise, parametric analyses (Pearson's r for correlation, Student's *t* test for comparison of groups means) were used and values were expressed as mean ± SD.

Total HDMA exposure to *Dermatophagoides pteronyssinus* was determined by adding up the HDMA exposure to *Der* p I/g and *Der* p II/g. When the HDMA concentration was below detection level, the detection level (5 ng/g) was used for calculations. In some locations, we found some dust samples containing HDMA levels below the detection level (*Der* p I: living room n = 4, bedroom n = 6, classroom n = 12; *Der* p II: living room n = 3, bedroom n = 4, classroom n = 16; *Der* p I and II: living room n = 1, bedroom n = 4, classroom n = 4). Association of type of floor-covering with HDMA exposure (log) was assessed with analysis of variance (ANOVA). Multiple regression analysis was performed on the PEF amplitude after withdrawal of ICS (dependent variable) with the following as independent variables: age (months), histamine responsiveness ($\log_2 PC_{20}$), log total HDMA exposure, ETS exposure, exposure to pets, and the interaction of ETS with PC_{20}.

A p value of 5% was considered as statistically significant. All analyses were performed with SPSS/PC+ package, version 4.0 (SPSS Inc, Chicago, Il, USA).

Results

Clinical Characteristics of the Subjects

Clinical characteristics are presented in Table 1. Frequencies of the reported symptoms were low, cough being the most common. FEV_1 deteriorated significantly after withdrawal of ICS (during ICS: 92.7 ± 12.4%, after withdrawal of ICS: 88.3 ± 13.3%, $p < 0.05$). FEV_1/IVC deteriorated, but not significantly, after withdrawal of ICS (during ICS: 83.1 ± 6.8%, after withdrawal of ICS: 81.3 ± 8.9%). Absolute PEF values during treatment with ICS showed a circadian pattern, with highest values in the afternoon (noon: 317 ± 10 l/min) and lowest values during the night (midnight: 282 ± 11 l/min). Absolute PEF values after withdrawal of ICS at midnight and 4:00 a.m. decreased significantly (midnight: 282 ± 11 l/min to 267 ± 10 l/min, $p = 0.05$; 4:00 a.m.: 283 ± 11 l/min to 269 ± 10 l/min, $p = 0.05$). The PEF amplitude correlated, both during and after withdrawal of ICS, significantly with daytime and nighttime symptoms during ICS. The PEF amplitude after withdrawal of ICS correlated inversely with PC_{20} and positively with the eosinophil count (Table 2).

Exposure to Exogenous Stimuli

Twenty-six (47%) children were exposed to ETS because of smoking habits from their mother and/or father. Maternal smoking was reported in four families (7%), paternal smoking in nine families (16%), and both parents smoked in 13 families (24%). Pets were present in 23 (42%) households: dog (n = 8; 15%), cat (n = 5; 9%), dog and cat (n = 6; 11%), guinea pig (n = 3; 5%) and a bird (n = 1; 2%). From 25 children, dust samples of the mattresses were collected as well. These 25 children did not differ from the other 30 children with regard to their clinical variables. Of these 25, 14 (56%) were exposed to an HDMA level higher than 10,000 ng/g of fine dust in their mattresses. Eleven children (11/25 = 44%) were exposed to HDMA levels in their mattresses below 10,000 ng/g of fine dust.

Exposures to *Der* p I and *Der* p II in each location and per type of floor-covering are presented in Figure 1. Mattresses contained the highest HDMA exposure levels, and classrooms had lowest HDMA exposure ($p < 0.001$). In 80% of the cases (n = 20), mattresses had the highest HDMA exposure levels compared with the other locations (living rooms: 12% [3 of 25], bedrooms: 8% [2 of 25]). A significant difference in HDMA exposure was observed between the types of floor-covering in living rooms and classrooms. The lowest values were measured on smooth floor-coverings and highest on smooth floor-coverings with a small carpet on it.

No significant differences in HDMA exposure levels were found between households with and without pets. No significant correlations between HDMA exposure levels at any location and absolute humidity levels were found.

Relation of Exogenous Stimuli with PEF Amplitude

There were no significant correlations found between the magnitude of the PEF amplitude and HDMA exposures for each location separately or when expressed as all locations combined (Spearman's rho between -0.10 and 0.05 during ICS, and between 0.03 and 0.18 after withdrawal of ICS; $p > 0.05$). Children exposed to ETS, pets, or high HDMA levels (>10,000 ng/g of dust) in their mattresses or to combinations of these stimuli had significantly higher PEF amplitudes after withdrawal of ICS than the nonexposed children (Table 3). This was not found during ICS. Figure 2 shows that the mean PEF amplitude after withdrawal of ICS was higher with increasing numbers of different exogenous factors. The largest amplitude was present in those exposed to the combination of ETS, pets, and high HDMA in the mattress.

Multiple Regression Analysis on PEF Amplitude

The results of multiple regression analysis of PEF amplitude after withdrawal of ICS (R^2 = 48.4%, $p = 0.000$) are shown in Table 4. Exposure to ETS, pets, and HDMA, the interaction of exposure to ETS with PC_{20}, PC_{20} and age were significantly contributing variables.

The interaction between PC_{20} and ETS indicates a larger PEF amplitude after withdrawal of ICS in children exposed to ETS when they had moderate to mild bronchial responsiveness (those with a PC_{20} higher than the median PC_{20} value [0.8 mg histamine/ml]). (PC_{20} > 0.8 mg/ml: no ETS 16.6% [7.3 - 33.2], with ETS 29.6% [3.9 - 52.1], $p = 0.05$; $PC_{20} \leq 0.8$ mg/ml: no ETS 26.8% [0.0 - 56.6], with ETS 31.9% [12.4 - 56.6], $p = 0.46$).

TABLE 1 Clinical characteristics of the patients

	During ICS	After withdrawal of ICS
Male / female	33 / 22	
Age, yrs	9.3 ± 1.7	
Daytime symptoms ▼		
Wheeze	0 (0 - 52)	0 (0 - 3)
SOB	1 (0 - 57)	0 (0 - 3)
Cough	5 (0 - 60)	0 (0 - 3)
Phlegm	0 (0 - 37)	0 (0 - 3)
Total symptom score	12 (0 - 206)	1 (0 - 11)
Nighttime symptoms ▼		
Wheeze	0 (0 - 49)	0 (0 - 3)
SOB	0 (0 - 48)**	0 (0 - 3)
Cough	2 (0 - 53)**	0 (0 - 3)
Phlegm	0 (0 - 15)	0 (0 - 2)
Total symptom score	5 (0 - 150)*	0 (0 - 11)#
FEV_1 % pred, %	92.7 ± 12.4	88.3 ± 13.3*
FEV_1/IVC, %	83.1 ± 6.8	81.3 ± 8.9##
PEF amplitude, %	26.4 ± 18.8	26.9 ± 14.7
PC_{20}, mg/ml	0.80 (0.02 - 15.7)	
Eosinophils, 10^6/l	610 ± 437	
Total IgE, IU/ml	379 (17 - 5,782)	
Specific IgE HDM, PRU/ml	38 (4 - 377)	

Values are expressed as mean ± standard deviation or median (minimum - maximum) depending on the skewedness of the distribution. SOB: shortness of breath, ICS: inhaled corticosteroids, HDM: house dust mite. Differences were calculated by Student's *t* test or Mann-Whitney *U* test depending on the skewedness of the distribution. ▼: during ICS symptoms were scored during 3 weeks, after withdrawal of ICS symptoms were scored on 1 day. **: p = 0.01 between daytime and nighttime symptoms; *: p = 0.02 between daytime and nighttime symptoms or FEV_1 during and after withdrawal of ICS; #: p = 0.06 between daytime and nighttime symptoms; ##: p = 0.09 during versus without ICS.

TABLE 2 Correlations between PEF amplitude during and after withdrawal of inhaled corticosteroids (ICS) and clinical parameters.

	PEF amplitude during ICS r or rho	PEF amplitude after withdrawal of ICS r or rho
Total symptoms daytime	0.43***	0.28*
Total symptoms nighttime	0.42***	0.26*
FEV_1 % pred ICS +, %	0.00	-0.21
FEV_1 % pred ICS -, %	-0.04	-0.21
Log_2 PC_{20}, mg/ml	-0.24#	-0.30*
Log eosinophils, 10^6/l	0.19#	0.30*
Log total IgE, IU/ml	-0.05	0.13
Log specific IgE HDM, PRU/ml	0.03	0.15

Correlation coefficients are Spearman's rho (symptoms) or Pearson's r (others) depending on the skewedness of the distribution. ICS +: during treatment with inhaled corticosteroids, ICS -: after withdrawal of inhaled corticosteroids. Significant correlation coefficients: *: $p < 0.05$, **: $p < 0.01$ ***: $p < 0.001$, #: $p = 0.08$.

TABLE 3 PEF amplitude (%) during and after withdrawal of inhaled corticosteroids (ICS)

	n	PEF amplitude during ICS	PEF amplitude after withdrawal of ICS
Total group	55	26.4 ± 18.8	26.9 ± 14.7
Non-ETS	29	20.6 (5.7 - 63.4)	19.4 (0.0 - 56.6)*
ETS	26	28.7 (10.7 - 99.0)	29.7 (3.9 - 56.6)
No pets	32	21.6 (5.7 - 83.5)	21.9 (0.0 - 56.6)*
Pets	23	23.4 (8.4 - 99.0)	31.4 (9.7 - 52.5)
Non-ETS, no pets	18	17.6 (5.7 - 63.6)	17.7 (0.0 - 56.6)**
ETS and pets	12	28.7 (11.3 - 99.0)	34.1 (12.8 - 51.4)
Mattress group	25		
Der p (I + II) < 10,000 ng/g	11	29.2 (7.4 - 63.6)#	21.4 (3.9 - 56.6)*
Der p (I + II) > 10,000 ng/g	14	33.3 (5.7 - 99.0)	35.5 (10.2 - 56.6)
Non-ETS, no pets and HDMA < 10,000 ng/g	5	29.9 (7.4 - 63.6)	21.4 (7.3 - 56.6)
ETS, pets and HDMA >10,000 ng/g	4	36.7 (28.7 - 99.0)	42.4 (37.8 - 51.4)

ETS: environmental tobacco smoke; n = number of children. Values are expressed as mean ± standard deviation or median (minimum - maximum) depending of the skewedness of the distribution and significant differences in PEF amplitude between exposed and non-exposed groups are done by Mann-Whitney *U* test. *: $p \leq 0.05$, **: $p = 0.01$, #: $p = 0.08$.

TABLE 4 Explaining model for the PEF amplitude (n = 55) after withdrawal of inhaled corticosteroids (R^2: 48.4%, p = 0.000)

	ß	p value
Constant	17.9	0.19
Pets in the household	9.8	0.004
Exposure to ETS	11.2	0.001
Log house dust mite exposure in living room, bedroom and classroom	7.5	0.006
Interaction between ETS and $\log_2 PC_{20}$	4.5	0.008
$\text{Log}_2 PC_{20}$	5.1	0.07
Age, months	-0.2	0.003

ETS: environmental tobacco smoke.

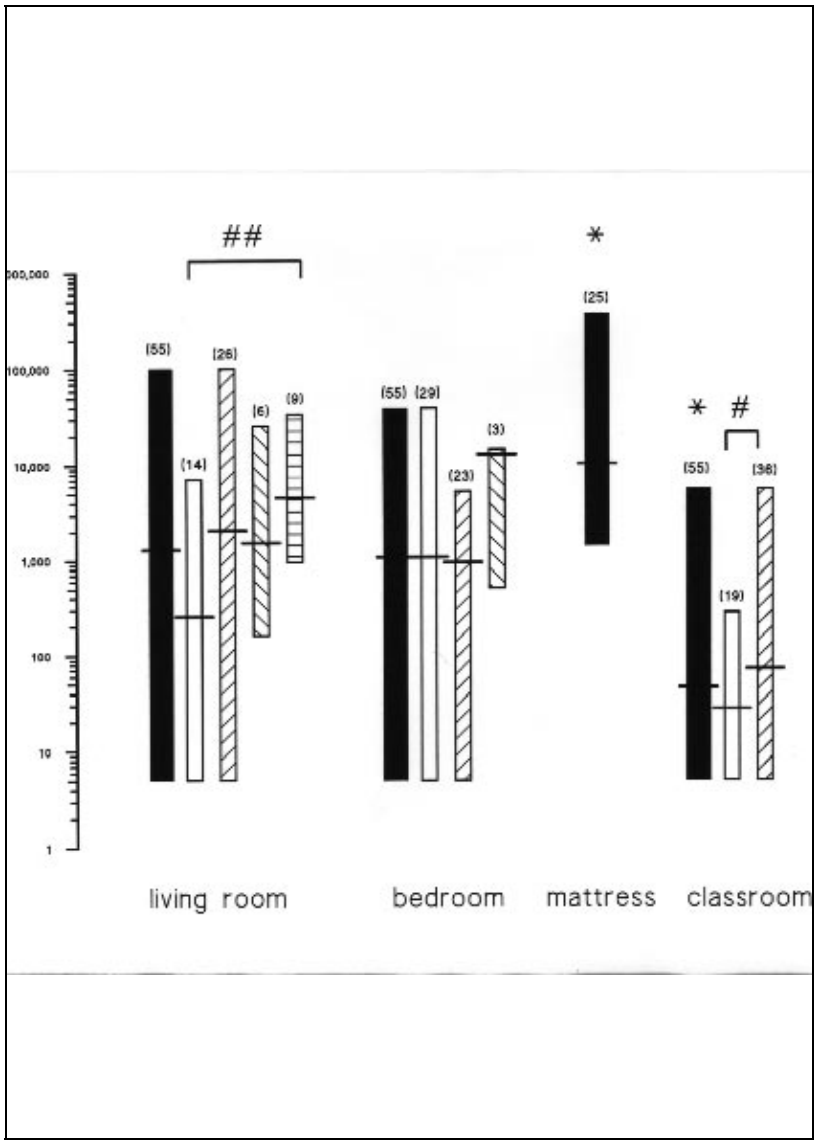

Figure 1 House dust mite allergen exposure to *Der* p I and *Der* p II per gram of fine dust in living rooms, bedrooms, mattresses, and classrooms (median value with ranges). Results are given per location for all samples (black bars) and per type of floor-covering: smooth floor-covering (open bars), synthetic wall-to-wall carpets (/// bars), woolen or cotton wall-to wall carpets (\\\ bars), smooth floor-covering with carpet on it (horizontal striped bars). The number of samples is given on top of each bar. *: significantly higher or lower ($p < 0.001$) compared with other type of floor-coverings. There was a significant difference in HDMA exposure per type of floor-covering (## living room, $p < 0.001$ and # classroom, $p < 0.05$; ANOVA).

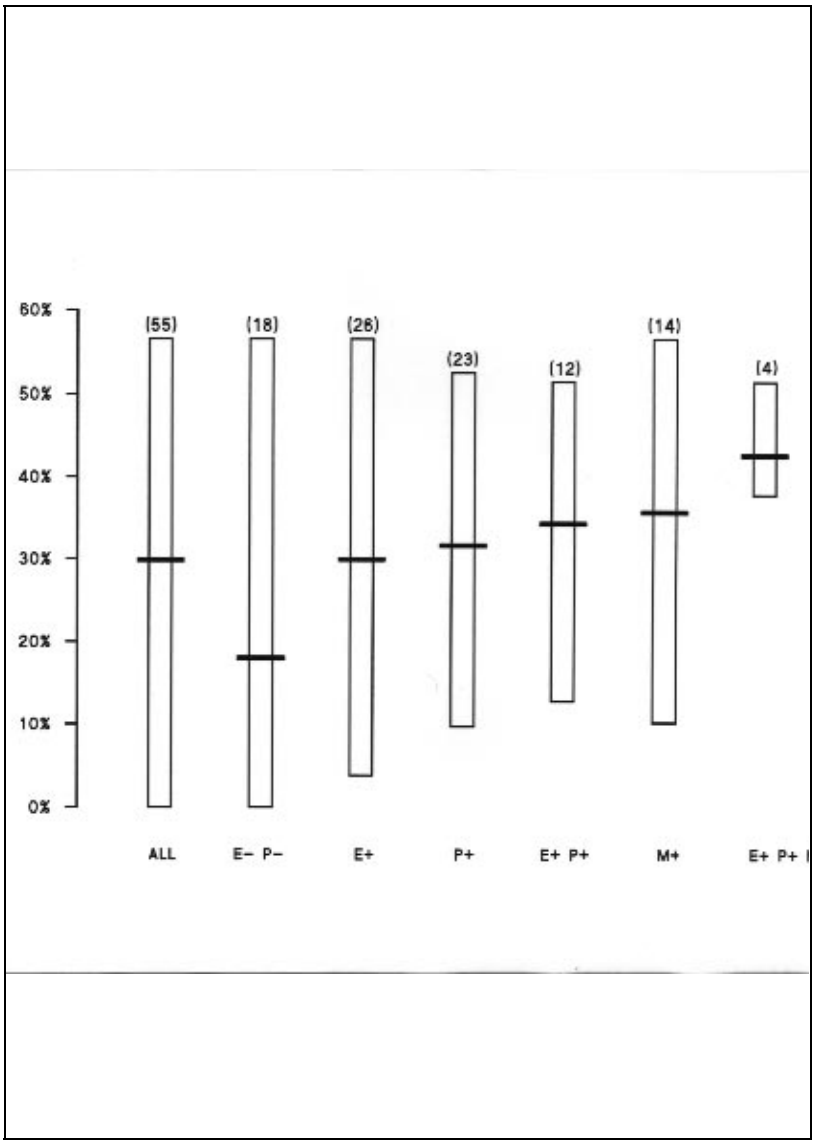

Figure 2 PEF amplitude after withdrawal of inhaled corticosteroids (ICS). Bars represent the results (median value with ranges) of all children. All = all children; E- P- = not exposed to environmental tobacco smoke (ETS) and pets; E+ = exposed to ETS; P+ = exposed to pets; E+ P+ = exposed to ETS and pets; M+ = exposed to high HDMA level (> 10,000 ng /g) in mattress; E+ P+ M+ = exposed to ETS; pets and high HDMA level in mattress. The number of samples is given on top of each bar.

Discussion

This study demonstrates a significant association between exposure to exogenous stimuli and an increased circadian PEF amplitude after withdrawal of inhaled corticosteroids (ICS) in allergic asthmatic children. Environmental tobacco smoke (ETS), pets and high exposure to house dust mite allergens (HDMA) in bedding contributed independently to a higher PEF amplitude after withdrawal of ICS. Multiple regression analysis showed that these exogenous stimuli explained 48.4% of the variation in PEF amplitude after withdrawal of ICS together with the interaction of ETS with PC_{20}, PC_{20} itself, and age. Although the PEF amplitude in the total group hardly increased after withdrawal of ICS, nocturnal PEF values (at midnight and 4:00 a.m.) decreased significantly. Finally, highest HDMA exposure levels were found in mattresses and on smooth floor-coverings with a carpet, lowest HDMA levels on smooth floor-coverings.

The results of our study are in agreement with epidemiologic studies suggesting that exposure to ETS may have adverse effects on children's pulmonary function. The lower pulmonary function values among schoolchildren were found to be associated with maternal smoking as a consequence of the combination of a persistent deficit related to earlier (including *in utero*) exposure and an additional deficit related to current exposure (14). This indicates that ETS has not only a short-term effect, but long-term exposure is even of greater importance. Frischer and associates (5) observed a negative effect of ETS on diurnal PEF variability in non-allergic asthmatic and normal children, but could not confirm this for allergic asthmatic children. They hypothesized that mothers tended to change their smoking habits subsequent to the development of disease in their allergic children. However, no data on the treatment in these asthmatic children were presented. Our study shows a larger effect of ETS after withdrawal of ICS. Thus, the lack of correlation in the study of Frischer and associates (5) can be explained when allergic children in the study used anti-inflammatory drugs, which is plausible given the consensus on their use in asthma treatment. We do now show that withdrawal of ICS results in a large PEF amplitude especially in those asthmatic children who are regularly exposed to ETS.

The relationship between ETS and bronchial responsiveness is conclusive (15,16). Even in young normal children, parental smoking contributes to increased bronchial responsiveness (17). O'Connor and coworkers (15) investigated the relationship between passive cigarette smoking and non-specific bronchial responsiveness in a population-based sample of children and young adults. They found a borderline significant relationship between bronchial responsiveness and maternal smoking in asthmatic young adults but not in similarly aged non-asthmatics, despite the occurrence of lower FEV_1 and FEF_{25-75} values in this group. In our asthmatic children, we did not measure bronchial responsiveness to an inhaled bronchoconstricting agent before and after withdrawal of ICS. We found a

significant interaction of ETS and PC_{20} with regard to PEF amplitude after withdrawal of ICS: children with mild to moderate bronchial responsiveness had a larger PEF amplitude when exposed to ETS. It is widely known that cessation of ICS is associated with a deterioration of bronchial responsiveness, in which airway inflammation is thought to be the underlying mechanism. It seems attractive to hypothesize that the interaction of ETS and bronchial responsiveness during ICS occurs via increased inflammation, ultimately resulting in higher PEF variability and unstable asthma, as suggested by the larger PEF amplitude 6 d after withdrawal of ICS in the ETS-exposed group. It may well be that the effect of ETS is minimal when airway inflammation is extremely active as can be expected in children with very severe bronchial responsiveness towards exogenous stimuli.
Children with pets in their households had a larger increase in their PEF amplitude after withdrawal of ICS. Allergies to animals in households, such as cats and dogs, are common. Antigens from virtually any animal, including exotic species, may produce allergic symptoms (6). Notwithstanding the exclusion of children with an allergy to cats and dogs, we found that the exposure to pets in the household negatively affected the PEF variation after withdrawal of ICS. An explanation for this observation in children with a solitary allergy to HDM may be that HDMA are more airborne due to air disturbance of pets, resulting in a higher airway provocation. Swanson and colleagues (18) described a good correlation between airborne and settled dust samples, but they did not look for the influence of air disturbance by pets. Another explanation for the larger PEF amplitude after withdrawal of ICS in the children with pets in their household may be that the development of specific IgE to cats and dogs is slower than the development of clinical symptoms. It is also possible that the influence of pets and ETS on PEF amplitude and bronchial responsiveness is due to cross-reactive priming, by which exposure to non-specific antigens leads to priming of bronchial responsiveness and PEF amplitude. The magnitude of the effects seen in this investigation are physiologically small but plausible because of chronic low-dose exposure to multiple potential exposure factors.
Children exposed to high HDMA levels in their mattresses (>10,000 ng/g of dust) had a significant higher PEF amplitude after withdrawal of ICS than those exposed to lower HDMA levels in their mattresses. The recognition that HDM faecal pellets are the source of the antigen causing HDM allergy has led to the suggestion that intensive contact with mites in bedding might precipitate airway obstruction at night (19). The mattress was the highest source of HDMA exposure in 80% of our population. This percentage is comparable with other observations in children (60 to 73%) (20), and much higher than in adults (21). Gervais and associates (3) previously described an increased reactivity to HDM at night. Our current observations indicate that, together with the well-known increased responsiveness to HDM overnight, exposure to high HDMA levels from mattresses may contribute to an increased nocturnal airway obstruction. Opposite to our findings, other studies

found that HDMA exposure from mattresses was not significantly higher than in the other locations. An explanation for the discrepancy with our findings might be that the HDMA exposure in the living rooms, bedrooms and classrooms in our study are low and lower than in most other reports (20,22,23).
Smooth floor-coverings generally contain the lowest HDMA levels. An interesting observation in our study is that the highest HDMA levels were found on smooth floors with a small carpet on it, being even higher than in woolen carpets. Zock and colleagues (24) showed higher diurnal PEF variations with higher HDMA levels collected from carpeted floors. Their results as well as ours emphasize the importance of the contribution of HDMA exposure to the subsequent development of nocturnal airway obstruction in HDM-allergic individuals. Moreover, results of both studies indicate that HDMA avoidance measures in HDM-allergic children with nocturnal airway obstruction should be focussed on high HDM sources such as mattresses and carpets, both wall-to-wall and small ones.

We conclude that exposure to exogenous factors such as ETS, pets, and high HDMA levels in mattresses contribute independently to an increased PEF amplitude after withdrawal of ICS in allergic asthmatic children. Finally, ETS seems especially to worsen PEF variability in children with mild to moderate bronchial responsiveness.

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CHAPTER 5

SEASONAL VARIATIONS IN HOUSE DUST MITE INFLUENCES THE CIRCADIAN PEAK EXPIRATORY FLOW AMPLITUDE

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Summary

The aim of the study was to investigate whether seasonal differences in house dust mite (HDM) allergen exposure influence the circadian peak expiratory flow (PEF) amplitude in asthmatic children. Asthmatic children (n = 25) with a solitary allergy to HDM were studied in spring and in autumn. All used inhaled corticosteroids (ICS) regularly. Six days after withdrawal of ICS PEF amplitude (every 4 h during 24 h, highest minus lowest as a percentage of day's mean value) was assessed. HDM allergen (HDMA) in living rooms, bedrooms and mattresses were collected. HDMA levels were not always highest in autumn. PEF amplitudes in spring and autumn did not correlate with HDMA levels in the same season. However, the seasonal difference in PEF amplitude (autumn value minus spring value) correlated positively and significantly with the seasonal difference in HDMA exposure levels from the mattresses (rho = 0.34, $p < 0.05$). Multivariate analysis showed that the seasonal difference in HDMA exposure in the mattress was the single parameter explaining seasonal difference in PEF amplitudes by 21.0% ($p = 0.02$). Our cross-sectional study showed a higher PEF amplitude not to be significantly associated with higher HDMA exposure in mattresses in a group of HDM-allergic asthmatic children. However, the change in HDMA exposure over seasons contributed significantly to the change in PEF amplitude after withdrawal of ICS in HDM-allergic asthmatic children.

Introduction

Natural indoor exposure to house dust mites (HDM) allergens is known to vary from season to season (1), although its variation is much less pronounced than exposure to pollen allergens. HDM inhalation in HDM-allergic individuals may result in an early and late asthmatic response, often followed by increased airway obstruction for many nights (2,3). Reduction of environmental allergens has been shown to improve the disease (4), and may result in a decrease of the circadian peak expiratory flow amplitude.

Little data are available with respect to the influence of a change in natural exposure levels of HDM allergens (HDMA) on clinical relevant variables. Although many clinicians in our part of the world have the impression that asthma deteriorates during autumn in many patients, the HDM season, it is not known whether seasonal variations in exogenous stimuli such as HDMA levels are associated with seasonal differences in PEF amplitude. In an earlier study we have found that exogenous factors such as HDMA exposure, environmental tobacco smoke and the presence of pets contributed to the magnitude of the circadian PEF amplitude measured in one season of the year (5).

In this study we further explored whether the seasonal difference in HDMA

exposure contributes to an increase in circadian PEF amplitude in asthmatic children with a mono-allergy to HDM.

Methods

Patients

Twenty-five asthmatic children (17 boys, 8 girls) aged 6 to 12 yrs were included. All were characterized by symptoms of asthma, increased total serum IgE, and specific IgE to HDM (RAST class ≥ 3, Pharmacia Diagnostics, Uppsala, Sweden). Children with other allergies proven by a positive RAST test were excluded in order to obtain a homogeneous population. All children had a forced expiratory volume in one second (FEV_1) of at least 70% of the predicted value, and increased bronchial responsiveness (histamine provocation concentration ≤ 8 mg/ml causing a fall of 20% or more in FEV_1 from baseline value). All children used inhaled corticosteroids (ICS) as maintenance medication, twice daily 200 or 400 µg, for at least 4 mo before the study and short-acting ß$_2$-adrenergic drugs when needed. None of the children used long-acting ß$_2$-adrenergic drugs. Routine standardized information with regard to reduction of environmental stimuli was given previously by pediatricians and/or nurses at our outpatient clinic. Acaricides, mattress encasings and dehumidifiers were not used in the investigated houses. Informed consent from all children and their parents was obtained, and the study was approved by the Medical Ethics Committee of our hospital.

Study Design

The same measurements were performed in all children both once in spring (March to May) and once in autumn (August to November). Children were included either in the spring or in autumn. To complete the group, children were included during three consecutive years. Seasonal differences were defined as the autumn value minus the spring value.

Daytime and nighttime symptoms were recorded in a 3-wk period during regular treatment with ICS and on the sixth day after withdrawal of ICS. At the end of the 3-wk period, a home and school visit was made to collect house dust from floors of living rooms and bedrooms and from mattresses. Temperature and relative humidity were measured in each location to obtain absolute humidity (gram water vapor per kilogram of dry air). A histamine challenge test was performed and a blood sample was drawn during ICS use at the outpatient clinic to determine bronchial responsiveness, the eosinophil count, total IgE, and specific IgE to HDM. FEV_1 during and at the sixth day after withdrawal of ICS was performed at the outpatient clinic. The circadian PEF amplitude (every 4 h during 24 h, expressed as highest minus lowest PEF value expressed as a percentage of the day's mean value) was obtained at home on the sixth day after withdrawal of ICS.

Clinical Characteristics

SYMPTOMS: Symptoms of cough, wheeze, dyspnea and phlegm production during the day and at night were recorded in a diary on a 4-point scale (0: no symptoms, 1: mild, 2: moderate, 3: severe) (6). Daytime and nighttime symptoms were recorded and added up during the 3-wk period to obtain the total symptom score at daytime and nighttime during ICS. Symptoms were also recorded during daytime and nighttime on the sixth day after withdrawal of ICS.

LUNG FUNCTION AND BRONCHIAL RESPONSIVENESS: Short-acting $ß_2$-adrenergic drugs were withheld 8 h before the measurements. PEF measurements were performed at home, every 4 h during 24 h, in an upright position with a mini-Wright peak flow meter to assess the circadian PEF amplitude. The best of three efforts was used for statistical analysis. FEV_1 was measured with a water-sealed spirometer (Lode BV, Groningen, the Netherlands). At least three reproducible values (i.e. < 5 percent difference among the recordings) were obtained; the highest was used in the analysis. Airway histamine challenge tests were performed during ICS with a gauged DeVilbiss 646 nebulizer (DeVilbiss, Somerset, MA, USA), with an output of 0.13 ml/min according to the modified method of Cockcroft and colleagues (7). A 0.9% phosphate-buffered saline solution and doubling histamine concentrations from 0.03 to 16 mg/ml were inhaled for two minutes during tidal breathing, with the nose clipped, at 5 min intervals, until FEV_1 had fallen by at least 20% from the initial value. The exact provocation concentration of histamine that induced a 20% fall in FEV_1 (PC_{20}) was assessed by a log-dose response curve.

Laboratory Investigations

Total IgE and specific IgE were quantified using an enzyme immunoassay procedure (Pharmacia Diagnostics), and expressed in international units (IU) per milliiter, and Phadebas RAST units (PRU) per milliliter, respectively.

House Dust Mite Allergen Exposure

All dust samples were obtained by the same technician, using a vacuum cleaner (Phillips type T580, 1100 W). For every location we used a different double-walled disposable paper bag (8), and a special vacuum cleaner filter for the mattresses (ALK filter device; surface, 38 cm^2, pore size, 6 µm; ALK, Hørsholm Denmark). Dust was collected from the total area of the location in order to obtain a representative sample. The total amount of fine dust of each floor sample was measured after filtering with a 355-µm aperture sieve. Each sample was analyzed for the amount of HDMA (*Der* p I and *Der* p II) per gram of fine dust according to the WHO International standards (9,10). After extraction of the dust samples, HDMA was analyzed by sandwich immunoassay using monoclonal antibodies (1).

Statistical Analysis

FEV_1 values were expressed as percentage of the predicted value (% pred) (11). PC_{20} values were used after logarithmic transformation (base 2), since these reflect doubling doses and a normalized distribution. In subjects who did not reach a 20% fall in FEV_1 after the maximum exposure of 16 mg/ml histamine (spring n = 4), PC_{20} was considered to be one doubling doses higher (32 mg/ml). Total IgE and specific IgE HDM were logarithmically transformed (base 10) to normalize the distribution. Skewedness of distributions was assessed with a Kolmogorov-Smirnov test. If a p value < 0.05 was obtained, nonparametric techniques (Spearman's rho for correlation, Mann Whitney U test to compare group means) were applied to analyze the data, values being expressed as median (minimum to maximum). Otherwise, parametric analyses (Pearson's r for correlation, Student's *t* test for comparison of groups means) were used and values were expressed in mean ± SD. Total HDMA exposure to *D. pteronyssinus* was determined by adding up the HDMA exposure to *Der* p I/g and *Der* p II/g. When the HDMA concentration was below detection level, the minimum detection concentration (0.01 µg/g for *Der* p I and *Der* p II) was used for calculations. Seasonal differences in clinical parameters were defined as the autumn value minus the spring value. Multiple regression analysis was performed to obtain a significant model for the seasonal difference in PEF amplitude after withdrawal of ICS as the dependent variable with the seasonal difference in HDMA exposure in the mattress (< -5 µg/g, -5 up to +5 µg/g and > 5µg/g of fine dust) as the independent variable. A p value less than 5% was considered as statistically significant. All analyses were performed with SPSS/PC+ package, version 4.0 (SPSS Inc, Chicago, IL, USA).

Results

Clinical Characteristics

Clinical characteristics of the children are presented in Table 1. Symptom scores, FEV_1, and PEF amplitude were not significantly different between the spring and autumn season. Mean PC_{20} histamine was significantly lower in autumn ($p < 0.05$) than in spring. The total number of eosinophils increased significantly during autumn ($p < 0.05$). PEF amplitudes, total IgE, and specific IgE to HDM were not significantly different between the two seasons.

Seasonal Exposure to Exogenous Stimuli

There was a significant seasonal difference in HDMA exposure due to a higher HDMA exposure in the autumn (Table 1). No significant correlation was found between the magnitude of HDMA exposure and the level of absolute humidity. Smoking habits, presence of pets and floor-coverings did not changed during the study period.

PEF amplitude after withdrawal of ICS

PEF amplitude after withdrawal of ICS in spring and autumn did not significantly correlate with the level of HDMA exposure in the same season (Table 2). However, PEF amplitude correlated significantly and inversely with PC_{20} in autumn (Table 2) and positively with daytime symptoms after withdrawal of ICS in the spring (rho = 0.47; p = 0.01).

Though highest HDMA levels and PEF amplitudes were expected to occur in the autumn, this was not the case in all children (living room n = 7, bed room n = 5, mattress n = 8). Therefore, we calculated whether correlations existed between the seasonal difference in PEF amplitude (autumn value minus spring value) and the other clinical variables.

The seasonal difference in PEF amplitude after withdrawal of ICS correlated significantly and positively with the seasonal difference in total symptom score at night during ICS (rho = 0.44; p = 0.02), inversely with the seasonal difference in FEV_1 after withdrawal of ICS and positively with the seasonal difference both in total HDMA exposure and in mattresses (Table 2). Figure 1 shows that children with a higher seasonal difference in HDMA exposure in the mattresses had higher seasonal differences in PEF amplitude after withdrawal of ICS. Since not all children had the highest HDMA exposure level in the autumn, the Figure also shows negative seasonal differences in HDMA levels and PEF amplitudes.

A multiple regression analysis on the PEF amplitude after withdrawal of ICS showed a significant explaining model (R^2 = 21.0%, p = 0.02) with the seasonal difference in HDMA in the mattress as the only significant contributing variable (Table 3).

TABLE 1 Patient characteristics in spring, autumn and the seasonal differences in clinical parameters (autumn value minus spring value) (n = 25)

	spring	autumn	seasonal difference
FEV_1 % pred +, %	95.8 ± 10.6#	93.7 ± 12.8	-0.6 ± 11.8
FEV_1 % pred -, %	89.7 ± 12.0	87.8 ± 14.0	1.0 ± 13.0
Log_2 PC_{20}, mg/ml	1.38 ± 2.56	0.34 ± 2.23	-1.04 ± 2.19*
Geometric mean PC_{20}, mg/ml	2.60	1.27	0.49
PEF amplitude -, %	23.6 ± 19.1	29.6 ± 15.5	0.7 ± 18.5
Eosinophils, 10^6/l	374 ± 277	561 ± 544	183 ± 424*
Total IgE, IU/ml	315 (20 - 2830)	318 (17 - 1529)	54 (-1379 - 355)
Specific IgE HDM, PRU/ml	28 (1 - 162)	42 (1 - 377)	10.7 (-138 - 215)
Der p I, II in LR, µg/g	0.4 (n.d. - 14.6)	1.2 (n.d. - 24.1)	0.9 (-1.1 - 14.2)**
Der p I, II in BR, µg/g	0.5 (n.d. - 4.1)	0.8 (n.d. - 14.6)	0.3 (-3.3 - 13.0)**
Der p I, II in MA, µg/g	6.1 (0.9 - 115.6)	10.1 (1.4 - 368.8)	1.4 (-111 - 341.8)
Der p I, II total, µg/g	8.6 (1.0 - 119.8)	19.6 (1.6 - 370.0)	9.6 (-112 - 342.9)*

Values are expressed as mean ± standard deviation or median (minimum - maximun) depending on the skewedness of the distribution. +: during inhaled corticosteroids, -: after withdrawal of inhaled corticosteroids, pred: predicted, n.d.: not detectable. Total = *Der* p I and II of living rooms (LR), bedrooms (BR), and mattresses (MA). p: significant seasonal difference (Student's *t* test or Mann-Whitney *U* test, depending on the skewedness of the distribution), *: $p \leq 0.05$, **: $p \leq 0.01$, #: FEV_1 % pred + versus - :$p < 0.001$.

TABLE 2 Correlations between PEF amplitude after withdrawal of inhaled corticosteroids in spring, autumn and seasonal difference (autumn value minus spring value), with clinical parameters and HDMA exposure in the corresponding period

	PEF amplitude after withdrawal of inhaled corticosteroids		
	spring (r or rho)	**autumn** (r or rho)	**seasonal difference** (r or rho)
FEV_1 % pred -, %	-0.05	-0.03	-0.36*
Log_2 PC_{20}, mg/ml	-0.20	-0.45**	0.12
Eosinophils, 10^6/l	0.29	0.22	0.07
Total IgE, IU/ml	-0.03	0.13	-0.07
Specific IgE HDM, PRU/ml	-0.04	0.15	0.05
Der p I, II in LR, µg/g	-0.27	0.20	-0.04
Der p I, II in BR, µg/g	-0.05	-0.09	0.28
Der p I, II in MA, µg/g	0.12	0.20	0.34*
Der p I, II in total, µg/g	0.17	0.11	0.35*

-: after withdrawal of inhaled corticosteroids, pred: predicted. Total = *Der* p I and II of living rooms (LR), bedrooms (BR), and mattresses (MA). Correlation coefficients are performed with parametric (r) or non-parametric (Spearman's rho) depending on the skewedness of the distribution. *: $p < 0.05$, **: $p < 0.01$.

TABLE 3 Multiple regression model for the seasonal difference in PEF amplitude after withdrawal of inhaled corticosteroids (R^2 = 21.0%, p = 0.02)

	ß	p value
Constant	-25.5	0.03
Seasonal difference in HDMA exposure in the mattress	11.7	0.02

HDMA: house dust mite allergen.

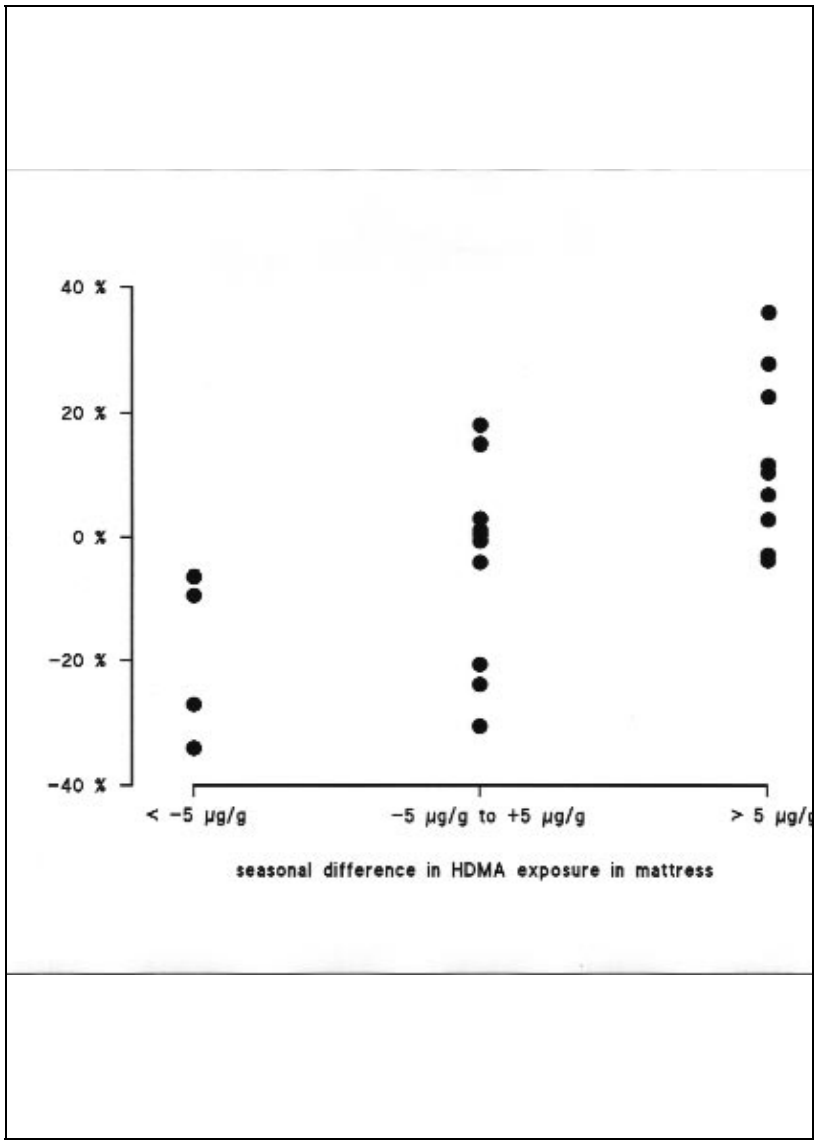

Figure 1 Seasonal difference (autumn minus spring value) in house dust mite allergen (HDMA) exposure of *Der* p I and *Der* p II (µg/g) in mattress and PEF amplitude after withdrawal of inhaled corticosteroids (ICS). Seasonal difference in HDMA exposure is divided in 3 categories (< -5 µg/g, -5 up to +5 µg/g and > 5µg/g of fine dust.

Discussion

This study shows that the absolute level of house dust mite (HDM) allergen exposure is not significantly correlated with the height of the PEF amplitude neither in autumn nor in spring. An important finding is that a larger seasonal change in HDM allergen (HDMA) exposure is associated with a larger change in circadian PEF amplitude. PEF amplitude at a given point in time appears to be determined by more factors than solely the amount of allergen an individual is exposed to. Furthermore our data show that an increase in HDMA exposure in the mattress over seasons enhances an individual's PEF variability.

Our study stresses the fact that mattresses are quantatively the most important source for HDM which affects PEF variability particularly (5,12). In a larger group of HDM-allergic asthmatic children we have previously shown that the occurrence of environmental tobacco smoke, the presence of pets, and high HDMA exposure levels together determine the PEF amplitude in one season to a large extent. This study extends this observation in that changes in exposure levels from the mattresses between seasons are clinically relevant, since they explained 21% of the differences in PEF amplitude between seasons.

Studies from different parts of the world found a seasonal variation in HDMA levels, with highest mean HDMA exposure levels in the autumn months (1,13-16). Our overall results are in accordance with these observations. However, these studies and our results indicate that the variation in exposure levels between seasons can be very small (1,16). Kalra and coworkers observed a statistically significant difference between the seasons (16), but considered the magnitude of the intra individual changes between the seasons small and not of clinical importance. They only measured HDMA concentrations and did not investigate the effect of changes in allergen exposure levels on clinical variables. Our study points to the fact that even relatively small changes in HDMA exposure levels are of clinical relevance for individual patients.

Other studies have emphasized the influence of HDMA exposure levels on clinical variables with regard to disease severity. Zock and colleagues (17) showed in a cross-sectional study that a higher diurnal PEF amplitude correlated with higher HDMA levels collected from carpeted floors. More evidence that HDMA exposure from mattresses is important comes from HDM reduction studies. Ehnert and associates (12) compared the effect of treatment of mattresses with an acaricide and mattress encasements, in allergic asthmatic children. They observed a reduction in the degree of bronchial responsiveness in the group that was treated with mattress encasements. Our data support these findings since we show that spontaneous variations in mattresses HDMA exposure are reflected by changes in circadian PEF variability.

Our observations confirm the findings of an earlier study in HDM-allergic adult patients with asthma (1), in which the increase in HDMA exposure level in autumn

coincided with an increase in bronchial responsiveness. We also found an increase in bronchial responsiveness together with an increased number of blood eosinophils in the autumn months, suggesting that the inflammatory process in the lungs is enhanced in this period of the year. The relationship between increased HDMA exposure levels on one hand, and increased bronchial responsiveness and increased blood eosinophils on the other hand is very likely a causal one since all our children were only allergic to HDM and children with respiratory infections were excluded. However, we did not find this association for PEF variability. Many investigators have suggested that PC_{20} and PEF variability represent both asthma instability and therefore can be used interchangeably. The discrepancy between seasonal changes in PC_{20} which were not reflected in changes in PEF amplitude indicate that both expressions of bronchial lability provide different information on the actual disease state as has been shown in earlier studies by other investigators (18-20).

In summary, we have found measurements of HDMA at a single point of time is not associated with PEF variability in the same season for all individuals. Moreover, we found that seasonal differences in PEF amplitude after withdrawal of ICS correlate significantly and positively with, and are explained to a large extent by the seasonal difference in HDMA exposure in mattresses. Our results amplify the general opinion that reduction of HDMA levels in bedrooms of HDM-allergic children, and especially in mattresses, contributes an essential aspect of the management of asthma.

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CHAPTER 6

MITE-SPECIFIC IGE CANNOT BE USED AS A SURROGATE FOR MITE EXPOSURE

Letter to the editor

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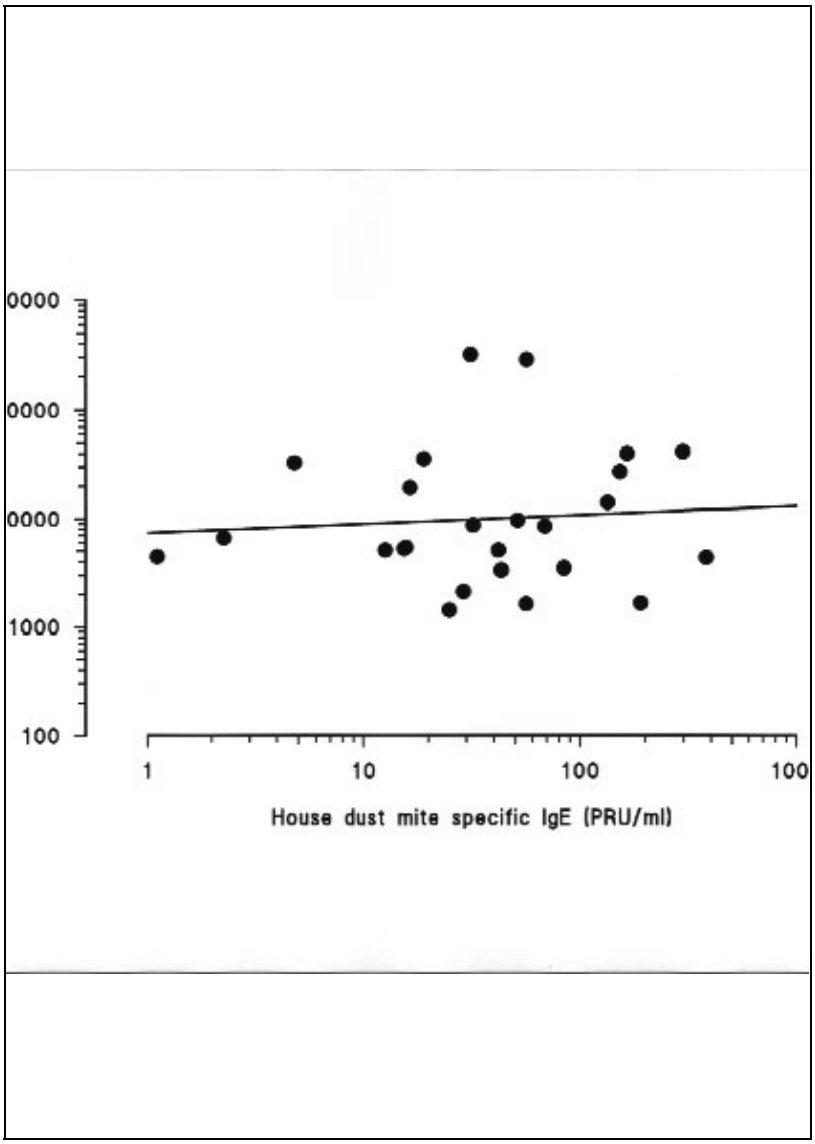
De Lovinfosse *et al.* recently (*Allergy* 1994: 49: 64-66) compared levels of mite-specific IgE (measured by RAST grade (0 - 6) and the level of mite group I allergen in mattresses (µg/g house dust mite) and found a significant correlation between both variables ($r = 0.57$, $p = 0.001$). Those with a RAST ≥ grade 3, had a 77% probability of being exposed to more than 10 µg/g mite allergen; those with RAST ≤ grade 2 had a probability of 77% of not being exposed to such high levels. From these findings, they concluded that serum mite-specific IgE could be used in routine prediction of mite exposure. We are concerned about the extrapolation of their findings for clinical and research purposes.

Therefore, we have reviewed data on 25 asthmatic children with an isolated allergy to house dust mite (HDM), all treated with maintenance therapy of inhaled corticosteroids. We have collected dust samples from the mattress from September to November. Median value of *Der* p I was 6,647 (range: 1,431 - 320,000 ng/g), median mite-specific IgE was 38 (range: 1.11 - 377 PRU/ml). We found *no* significant correlation between serum mite-specific IgE and *Der* p I exposure in the mattresses ($rho = 0.07$, $p = 0.76$, see Figure 1). Furthermore, no significant difference in HDM exposure was observed for those with RAST < 17.5 PRU/ml (n = 7) compared with those with RAST > 17.5 PRU/ml (n = 18) (5,422 (4,449 - 32,933) versus 8,665 (1,431 - 320,000 ng/g *Der* p I, $p = 1.0$). Comparable result was found for those with RAST < 3.5 PRU/ml (n = 2) compared with > 3.5 PRU/ml (n = 23).

As can be seen in Figure 1, there are even cases with high RAST to HDM but low (< 2,000 ng/g fine dust) *Der* p I level in their mattress. The results show once more that group findings and associations can not easily be extrapolated to individual predictions. Moreover, associations are probably affected by various factors, (e.g. therapy), as may be the case in our study.

Therefore, we strongly advice clinicians not to use the level of serum specific IgE as a surrogate of the level of HDM exposure in allergic asthmatic children.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House dust mite specific IgE (PRU/ml)

Figure 1

CHAPTER 7

LONG-TERM CIRCADIAN EFFECTS OF SALMETEROL IN ASTHMATIC CHILDREN TREATED WITH INHALED CORTICOSTEROIDS

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Abstract

The present study was set up to investigate whether salmeterol in asthmatic children already treated with inhaled corticosteroids (ICS) leads to a sustained bronchodilator effect and decreased bronchial responsiveness, both during the day and night. Furthermore, we investigated whether cessation of salmeterol leads to a rebound increase in bronchial responsiveness. Forty asthmatic children (aged 7 to 15 yrs) using ICS participated in a randomized, double-blind, parallel study. They received either twice daily 50 µg salmeterol xinafoate or placebo. Forced expiratory volume in one second (FEV_1) and provocation concentration of methacholine that caused a 20% fall in FEV_1 (PC_{20}) were measured at 4:00 p.m. and 4:00 a.m. at baseline and after 16 wk. The same measurements were performed at 4:00 p.m. at 8 h after the first dose, and after 1 and 8 wk. After cessation of the study drug, FEV_1 and PC_{20} were measured at 12 and 20 h, and after 1 wk. Overall mean FEV_1 from 1 to 16 wk of treatment was significantly higher in the salmeterol group than in the placebo group (difference, 4.9 ± 2.0%, p = 0.01). Evolution in time of FEV_1 did not differ significantly between the two groups (p = 0.09). Overall mean PC_{20} from 1 to 16 wk of treatment was not significantly higher with salmeterol than with placebo (difference, 0.7 ± 0.4 doubling dose [DD], p = 0.07); evolution in time of PC_{20} did not differ significantly between the two groups (p = 0.58). The increase of PC_{20} 8 h after the start of the study was significantly higher with salmeterol than with placebo (1.9 ± 0.3 DD versus 0.7 ± 0.3 DD, p < 0.01); significance disappeared at 1 wk. At 16 wk, the circadian variation (day minus night value) in FEV_1 was significantly smaller with salmeterol than with placebo (-0.9 ± 0.9% versus 2.2 ± 1.0%, p = 0.03); this was not the case for PC_{20}. No rebound worsening of FEV_1 or PC_{20} methacholine was observed after cessation of salmeterol. Sixteen weeks of treatment with salmeterol given to asthmatic children on regular ICS leads to a sustained bronchodilator effect and improved circadian variation in airway diameter. The initial significant decrease in bronchial responsiveness dropped below significance after 1 wk, and no overall additive beneficial effect of salmeterol on bronchial responsiveness was present. Finally, cessation of salmeterol after 4 mo of treatment does not lead to a rebound increase in bronchial responsiveness in asthmatic children treated with inhaled ICS.

Introduction

Despite maintenance medication with inhaled corticosteroids (ICS), a number of asthmatic children may have a pronounced circadian variation in airway diameter. This may cause interrupted sleep and early morning dyspnea, resulting in diminished concentration and school performance (1). A single inhalation of salmeterol induces bronchodilation for at least 12 h and may thus specifically

prevent the nocturnal dip in lung function when inhaled in the evening (2). Salmeterol is also known to provide well-maintained bronchodilation in long-term treatment (3-8). Moreover, it prevents airway narrowing induced by a number of bronchoconstricting stimuli (9-12). Therefore salmeterol seems a good choice as adjuvant therapy when ICS appear to be insufficient to stabilize asthma.
Recently it has been found that long-term monotherapy with salmeterol results in tolerance to the bronchoprotection against methacholine (13) and exercise (14), despite well-maintained bronchodilatory potential. Others could not confirm this observation of tolerance after long-term treatment with salmeterol (15). Further studies suggest that sudden withdrawal of short-acting ß-agonists may result in rebound increase in bronchial responsiveness (16,17).
ICS are known to reduce the fall in lung function and bronchial responsiveness and associated symptoms (18). Since salmeterol is effective for 12 h, it may be used as an adjunct to ICS when e.g. nocturnal symptoms remain. We therefore investigated in asthmatic children already treated with ICS whether 16 wk of treatment with salmeterol leads to a sustained bronchodilator effect and decreased bronchial responsiveness both during the day and night. Furthermore, we assessed whether cessation of salmeterol after 4 mo, when added to a regimen of ICS, also leads to a rebound increase in bronchial responsiveness.

Patients and methods

Subjects
Forty children with a history of allergic asthma (23 boys, 17 girls), aged 7 to 15 yrs, participated in this study. The children were selected from our outpatient department. All children were characterized by an increased total serum IgE and specific IgE to one or more common inhalation allergens (RAST ≥ 2, Diephuis Laboratories, the Netherlands), forced expiratory volume in one second (FEV_1) as percentage of the predicted value ≥ 70%, and increased bronchial responsiveness (histamine provocation concentration < 8 mg/ml (2 min tidal breathing inhalation; 5 min interval) causing a fall of 20% or more in FEV_1 from baseline (19). Symptoms were well-controlled for at least 4 mo before the study with maintenance ICS (twice daily 200 or 400 µg beclomethasone dipropionate rotadisk) and $ß_2$-adrenergic drugs if needed (salbutamol rotadisk). Informed consent from all children and their parents was obtained. The study was approved by the Medical Ethics Committee of the University Hospital of Groningen.

Study Design
The study had a randomized, double-blind, placebo-controlled, parallel design. There were three phases: a baseline period (screening and entry-day), regular treatment (16 wk), and cessation of treatment (1 wk). At the end of the baseline

period, the subjects were randomly assigned to one of the two groups, using either a dry powder inhaler (diskhaler) salmeterol xinafoate, 50 µg per inhalation, or a matched placebo at 8:00 a.m. and 8:00 p.m. First and last inhalations (after 16 wk of treatment) of the drug were supervised. Tests of FEV_1 and provocative concentration of methacholine that caused a 20% fall in FEV_1 (PC_{20}) were performed at 4:00 p.m. and thereafter at 4:00 a.m. at entry day. During regular treatment FEV_1 and PC_{20} were performed 8 h after the first inhalation of the first study medication (4:00 p.m.), after 8 wk of regular treatment (4:00 p.m.) and after 16 wk of regular treatment (4:00 p.m. and therafter at 4:00 a.m.). During cessation of treatment FEV_1 and PC_{20} were performed 12 and 20 h (8:00 a.m. and 4:00 p.m.) and 1 wk after the last study medication (4:00 p.m.). Time points of measurement (8 h after inhalation of the drug) were deliberately chosen since they reflect a more day-to-day control of the disease than measurements 1 h after inhalation, at the functional optimum of salmeterol. The time points of measuring bronchial responsiveness are within the period of action of salmeterol. Patients remained in hospital during the nocturnal measurements and the measurements on the day thereafter. Symptoms (wheezing, dyspnea, coughing, and phlegm production) (18) and rescue medication, during both day and night, were recorded (yes or no) for 1 wk during base-line period, the last wk of regular treatment period, and during cessation of treatment. Compliance of the study medication was checked after the study by counting the returned powder disks. The children were allowed to use a dry powder inhaler (diskhaler) delivering salbutamol with a maximum of 1,600 µg daily as rescue medication. Rescue medication was stopped for at least 8 h before the measurements.

Power analysis before the study estimated that 40 participating children were sufficient to detect 1 doubling dose (DD) improvement in PC_{20} methacholine.

FEV_1 and Methacholine Responsiveness

FEV_1 was measured with a water-sealed spirometer (Lode BV, Groningen, the Netherlands). At least three reproducible values (i.e. < 5% difference between the recordings) were obtained; the highest was used for analysis.

Bronchial challenge tests were performed with a gauged DeVilbiss 646 nebulizer (DeVilbiss, Somerset, MA, USA), with an output of 0.13 ml/min (19). A 0.9% phosphate-buffered saline solution and doubling methacholine-bromide concentrations ranging from 0.038 to 19.6 mg/ml (equipotent to 0.03 to 16 mg/ml methacholine chloride) were inhaled for 2 min, with the nose clipped, at 5 min intervals, until FEV_1 had fallen by 20% from baseline FEV_1. PC_{20} was assessed by linear interpolation of the last two points of the log concentration response curve. Short-acting $ß_2$-agonists were withdrawn 8 h before the measurements, ICS were continued.

Data Analysis

FEV_1 values were expressed as percentage of the predicted value (% pred) (20). PC_{20} values were used after logarithmic transformation (base 2). In subjects who did not reach a 20% fall in FEV_1 after the maximum dose methacholine, PC_{20} was considered to be one DD higher (39.2 mg/ml).

Distributions of variables with a Gaussian-shaped distribution were summarized by mean and standard deviation. Precision of estimated means and effects was presented by standard error of mean (SEM). For variables with a non-Gaussian-shaped distribution, the median and range (minimum, maximum) were used. Groups were compared by using the unpaired *t* test (Gaussian-shaped variables), the Mann-Whitney *U* test (non-Gaussian-shaped or ordinal variables), Chi-square test, or Fisher's exact test (categorical nominal variables). Treatment effects were estimated by applying rmANOVA to the relevant outcome variable, with treatment as between-patient grouping factor, time as within-patient factor, and the baseline measurement of the outcome variable as a fixed covariate. In a special analysis we added the interaction of time and treatment to the model to test constancy of the treatment effect over time, with time either as a factor or a quantitative trend variable. A p value less than 0.05 was considered to denote statistical significance. All analyses were performed with the packages SPSS/PC+ and BMDP (module 5V).

Results

Baseline period

One subject in the placebo group did not complete the study because of reasons unrelated to the study, but was included in the analyses up to his last visit (at 8 wk of treatment). Characteristics of the subjects in the baseline period were well in balance between the salmeterol and placebo groups (Table 1), daily dosages of inhaled corticosteroids were not significantly different between both treatment groups ($p = 0.06$). Daytime FEV_1 values of the total study group were significantly higher than at night (93.7 ± 14.3% and 90.3 ± 16.0% respectively, $p < 0.01$). This was not found for the PC_{20} values (geometric mean PC_{20} day, 1.0 mg/ml; night, 1.2 mg/ml, $p = 0.28$). Daytime values of FEV_1 and PC_{20} were higher but not significantly different in those who received 200 µg versus those who received 400 µg beclomethasone twice daily (FEV_1: 95.3 ± 13.1% versus 88.4 ± 17.6%, $p = 0.21$; geometric mean PC_{20} 1.2 mg/ml versus 0.5 mg/ml, $p = 0.18$). Compliance was comparable between the two groups.

Regular Treatment

LUNG FUNCTION: Overall mean FEV_1 (Figure 1) from 8 h after the first study medication up to 16 wk of treatment, was significantly higher in the salmeterol

group than in the placebo group, the difference being 5.4 ± 2.0% ($p < 0.01$); difference in time behavior of FEV_1 between the two groups, i.e., the course of FEV_1 during the study, was not significant ($p = 0.16$). Overall mean FEV_1 from 1 to 16 wk of treatment was significantly higher in the salmeterol group than in the placebo group, the difference being 4.9 ± 2.0% ($p = 0.01$). Difference in time behavior between the two groups was not significant ($p = 0.09$).
The increase in FEV_1 8 h and 1 wk after the start of the study (Figure 1) was significantly higher in the salmeterol than in the placebo group (5.7 ± 2.3% versus -1.4 ± 1.7%, p=0.02 after 8 h, and 4.7 ± 1.7% versus -3.3 ± 2.0%, $p = 0.01$ after 1 wk). Thereafter, differences between the two groups decreased and became insignificant (2.9 ± 2.0% versus -0.3 ± 2.5%, $p = 0.3$ after 8 wk, and 5.8 ± 2.6% versus 2.2 ± 2.1%, $p = 0.3$ after 16 wk).

BRONCHIAL RESPONSIVENESS: Overall mean PC_{20} (Figure 1) from 8 h after the first study medication, up to 16 wk of treatment, was significantly higher in the salmeterol group than in the placebo group (0.9 ± 0.4 DD, $p = 0.02$), when simultaneously tested over all time points. Overall mean PC_{20} from 1 to 16 wk of treatment was not significantly higher in the salmeterol group than in the placebo group (0.7 ± 0.4 DD, $p = 0.07$) when simultaneously tested over all but the first time points. Testing constancy of the treatment effect over time did not reach significance, neither over all time points ($p = 0.32$), nor over all but the first time points ($p = 0.58$). Using time as a quantitative trend variable (instead of factor) also did not lead to a significant time by treatment interaction.
The increase in PC_{20} 8 h after the start of the study was significantly higher in the salmeterol group than in the placebo group (1.9 ± 0.3 DD and 0.7 ± 0.3 DD, $p < 0.01$). Thereafter, differences in mean PC_{20} between the two groups became smaller and lost their statistical significance when tested seperately on each of the three later time points.
Both the salmeterol and the placebo group showed a significant increase in PC_{20} from baseline after 1 wk of treatment (salmeterol: 1.7 ± 0.3 DD, $p < 0.01$, and placebo: 0.9 ± 0.4 DD, p=0.04). This remained significant in the salmeterol group (1.2 ± 0.4 DD, $p = <0.01$ after 8 wk, and 1.1 ± 0.4 DD, $p = 0.01$ after 16 wk). This increase was not significant after 8 wk of treatment in the placebo group (0.6 ± 0.3 DD, $p = 0.11$), but it was significant after 16 wk of treatment (0.8 ± 0.4 DD, $p = 0.05$).
No significant differences in PC_{20} between those who received twice daily 200 or 400 µg beclomethasone could be observed within the salmeterol group.

CIRCADIAN VARIATION: Day-night differences in FEV_1 at baseline were comparable in the salmeterol and the placebo groups (2.0 ± 1.3% versus 4.7 ± 1.2%, $p = 0.12$), but at 16 wk this difference was significantly smaller with salmeterol than with placebo (-0.9 ± 0.9% versus 2.2 ± 1.0%, $p = 0.03$) (Figure 2).

Improvement in nocturnal FEV_1 during 16 wk of treatment with salmeterol was not significantly higher than with placebo (8.7 ± 2.5% versus 4.9 ± 2.1%, p = 0.25). The day-night differences in PC_{20} were both at baseline and after 16 wk of treatment not significantly different between the two study groups (p = 0.8) (Figure 2).

Cessation of Treatment

LUNG FUNCTION, BRONCHIAL RESPONSIVENESS: No significant differences in FEV_1 and PC_{20} methacholine values could be observed between the two study groups at 12 h, 20 h and 1 wk after cessation of the study medication (Figure 3), neither when compared to the placebo nor when compared to baseline values.

Subjective Aspects

Daytime and nighttime symptoms and the use of rescue medication (Table 2) were low and not sinificantly different between the salmeterol and placebo group during the baseline period, treatment period, and after cessation of the study medication.

TABLE 1 Characteristics of the study subjects by group, means (SD)

	ICS + SLM	ICS + PLA
No. of patients	20	20
Male/female	11/9	12/8
Age (yrs)	11.4 (2.4)	11.4 (2.8)
Weight (kg)	41.2 (10.6)	39.6 (13)
Height (cm)	149 (15)	147 (18)
ICS 400/800 µg daily	13/7	18/2
Duration of asthma (yrs)	8.9 (2.3)	7.7 (3.0)
Daytime		
FEV_1 % (pred)	93.6 (12.9)	93.8 (15.9)
FEV_1 / VC (%)	81.7 (8.7)	85.2 (9.0)
PEF (l/min)	365 (91)	369 (107)
Log_2 PC_{20} (mg/ml)	0.26 (1.97)	-0.25 (2.35)
Geometric mean PC_{20} (mg/ml)	1.20	0.84
Nighttime		
FEV_1 (% pred)	91.6 (13.5)	89.0 (18.4)
FEV_1 / VC (%)	80.2 (9.7)	82.5 (10.1)
PEF (l/min)	361 (90)	360 (106)
Log_2 PC_{20} (mg/ml)	0.49 (2.65)	0.06 (2.29)
Geometric mean PC_{20} (mg/ml)	1.41	1.04

ICS: inhaled corticosteroids; SLM: salmeterol; PLA: placebo. 400/800: number receiving 200 µg or 400 µg beclomethasone twice daily.

TABLE 2 Number of children with symptoms and use of rescue medication during baseline period, regular treatment period and during cessation of treatment

		Baseline period		**Regular treatment**		**Cessation of treatment**	
		ICS + SLM n = 20	ICS + PLA n = 20	ICS + SLM n = 20	ICS + PLA n = 20	ICS + SLM n = 20	ICS + PLA n = 20
Daytime							
Symptoms	wheeze	10	5	3	3	3	3
	dyspnea	14	8	4	5	5	3
	cough	16	12	8	7	10	5
	phlegm	11	7	4	4	4	2
Rescue medication		4	5	4	2	2	1
Nighttime							
Symptoms	wheeze	7	4	2	2	3	3
	dyspnea	12	9	5	5	7	5
	cough	16	12	7	7	10	3
	phlegm	9	7	4	3	5	3
Rescue medication		7	8	5	4	7	5

ICS: inhaled corticosteroids, SLM: salmeterol, PLA: placebo. No statistical significances (Chi-square tests) between ICS + SLM and ICS + PLA groups for each period ($p > 0.05$).

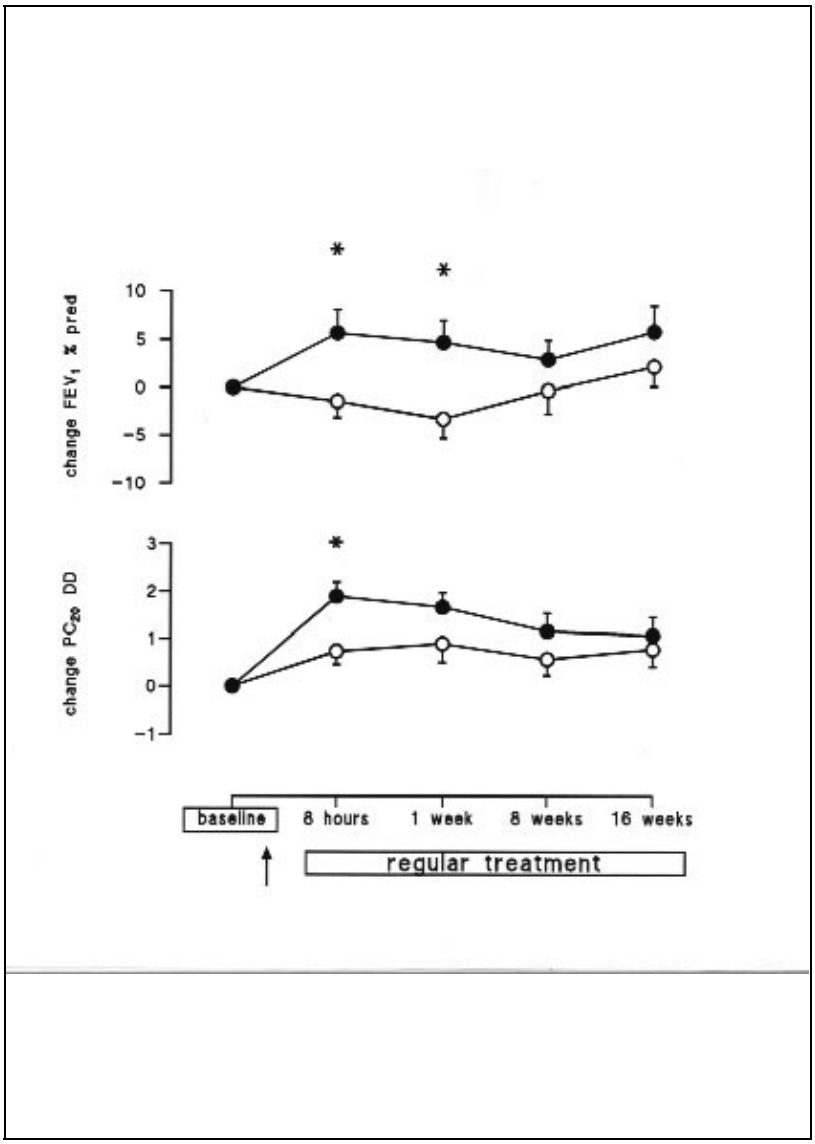

Figure 1 Mean (SEM) change in 4:00 p.m. FEV_1 % pred values (*upper panel*) and mean (SEM) change in 4:00 p.m. PC_{20} methacholine doubling doses (DD) values (*lower panel*) from baseline, of those who used inhaled corticosteroids with salmeterol (*closed circles*) and those who used inhaled corticosteroids with placebo (*open circles*). *: $p < 0.05$ between the two groups (*t* tests).

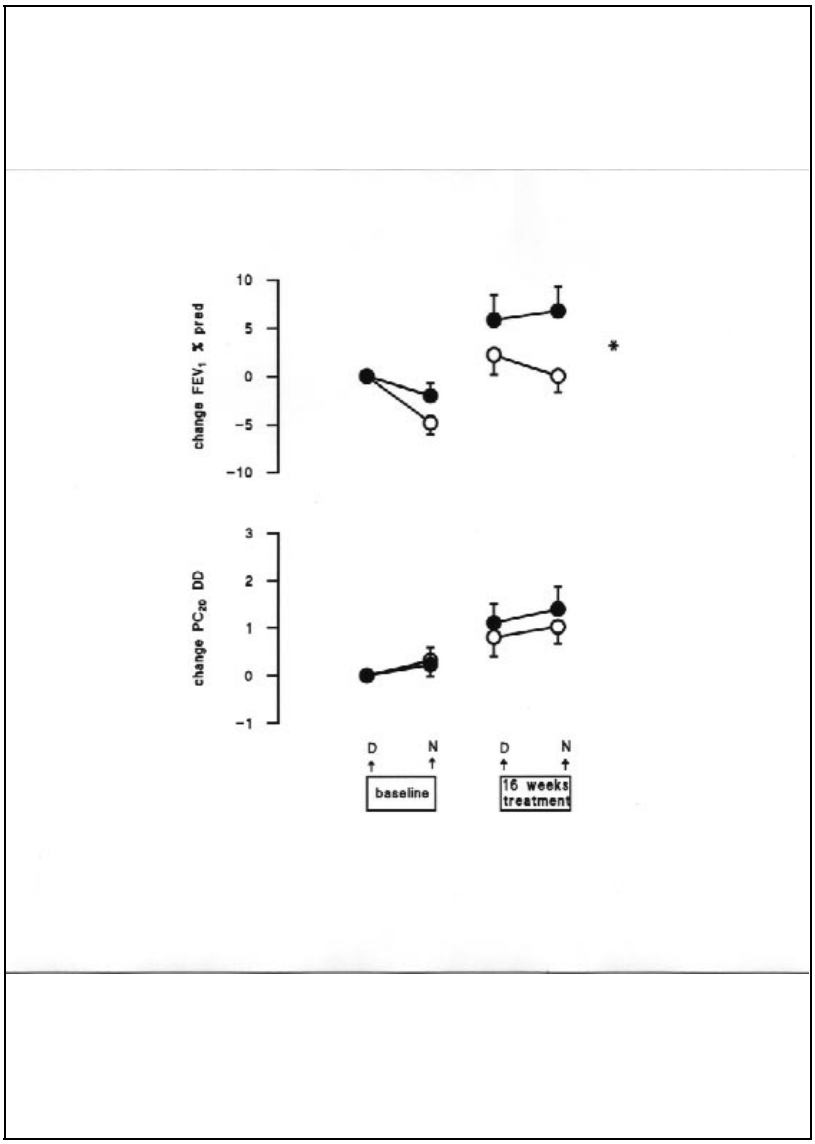

Figure 2 Mean (SEM) change in FEV_1 % pred values (*upper panel*) and mean (SEM) change in PC_{20} methacholine doubling dose (DD) values (*lower panel*) from baseline at 4:00 p.m. and 4:00 a.m. during baseline and after 16 wk of treatment of those who used inhaled corticosteroids with salmeterol (*closed circles*) and those who used inhaled corticosteroids with placebo (*open circles*). *: $p < 0.05$ for circadian variation between the two study groups at post-treatment period (*t* test).

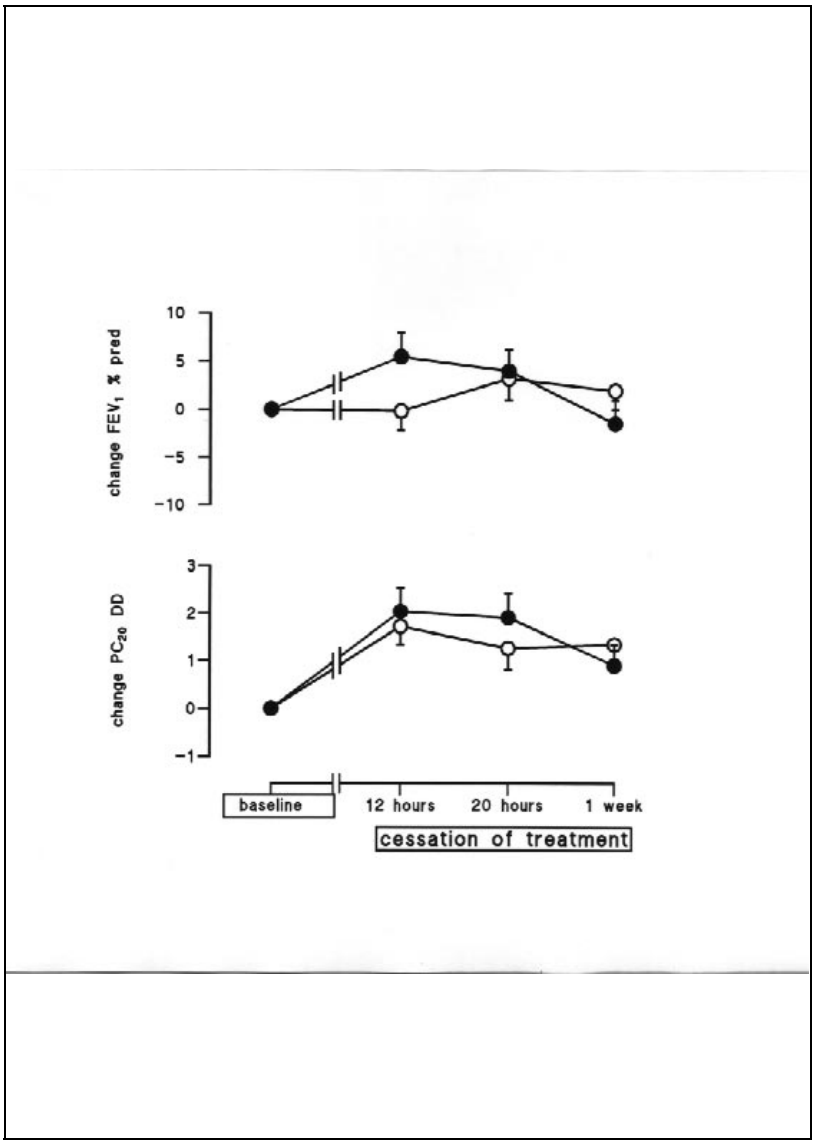

Figure 3 Mean (SEM) change in FEV_1 % pred values (*upper panel*) and mean (SEM) change in PC_{20} methacholine doubling dose (DD) values (*lower panel*) from baseline during wash-out period (12 h, 20 h and 1 wk after the last study medication) of those who used inhaled corticosteroids with salmeterol (*closed circles*) and those who used inhaled corticosteroids with placebo (*open circles*).

Discussion

This study in asthmatic children demonstrates that addition of salmeterol for 16 wk to maintenance treatment with inhaled corticosteroids (ICS) results in a sustained bronchodilator effect and a reduced circadian variation in FEV_1. Addition of salmeterol to ICS leads to a direct improvement of PC_{20} methacholine. Nevertheless, the mean change in PC_{20} after its first effect (from 1 to 16 wk of treatment with salmeterol) was not significantly different from placebo. This suggests that the combination of salmeterol and ICS does not seem to provide a clinically relevant supplementary gain with regard to bronchial responsiveness. Cessation of salmeterol after 4 mo of treatment showed no rebound worsening in FEV_1 or PC_{20} in this group of asthmatic children treated with ICS.

Several other studies in adults and children describe the maintained bronchodilating effect of salmeterol during long-term treatment (3-8,13,21,22). In some of these studies salmeterol has been investigated as monotherapy (8,13); in other studies part of the population used other anti-asthma medication, including ICS (3-7,21,22). In our study *all* children regularly used ICS. Our results show that salmeterol added to ICS provides a more pronounced bronchodilation than ICS alone, which effect did not appear to be significantly modified for 4 mo. We found that the overall mean FEV_1 value in the salmeterol group is significantly higher than in the placebo group. By including and testing a treatment by time interaction term in the model, a significant decrement of the bronchodilating effect of salmeterol could not be demonstrated, which is in agreement with d'Alonzo and colleagues (22) and Pearlman and colleagues (6).

Fitzpatrick and coworkers (23) showed the importance of a well-maintained nocturnal bronchodilation by salmeterol, as electroencephalography-controlled sleep quality improved significantly. Furthermore, salmeterol has been proven to reduce the number of nightly awakenings due to symptoms as well as sleep-related quality of life (23,24). ICS may also reduce nocturnal airway obstruction (25,26) along with reduction in diurnal PEF variation (3,4,6,21,22,27,28). Since salmeterol is effective for 12 h, it might also be used as an adjunct to ICS when nocturnal symptoms remain. Our results show for the first time that salmeterol exerts a beneficial effect, even in children with well-controlled asthma, both on daytime FEV_1, and on the circadian variation in FEV_1 when given on top of ICS. This indicates that salmeterol, in the long term, provides a more pronounced bronchodilating effect than a regimen with an ICS alone. The children included in this study, however, had a small circadian variation in lung function with values in the normal range; therefore, the opportunity to improve with salmeterol was limited. The clinical relevance of this study is that even in this group of children a sustained bronchodilator effect of salmeterol can be shown. However, salmeterol would not be the first choice of bronchodilator at present for this group of

asthmatic children.

We have observed an increase in PC_{20} during the 2 d the patients stayed in hospital in both the salmeterol and the placebo group. This improvement is most likely a consequence of staying in an allergen-free environment (29) and has been observed during earlier studies on nocturnal asthma (19,26). We found an increase in PC_{20} of almost one doubling dose during 16 wk of placebo inhalation, suggestive for an increased compliance with ICS. This shows once more the need for placebo-controlled investigations, when studying bronchodilating and bronchoprotective effects of drugs.

Wempe and coworkers (26) compared the effects of budesonide and bambuterol and found that both drugs improved bronchial responsiveness during the day and night. However, the effect of budesonide was stronger than that of the long-acting bronchodilator. We show in asthmatic children with only limited airway obstruction during both day and night, that salmeterol does provide limited improvement in FEV_1, most likely due to a ceiling effect. Even though they had limited airway obstruction and a small circadian variation in FEV_1, the children had moderate to severe bronchial responsiveness, which did not improve during the day or overnight when salmeterol was added to ICS. The latter lack of improvement cannot, however, be explained by a ceiling effect, since there was still enough room for improvement in PC_{20}.

Our patients were at the start of the study well controlled, with a mean FEV_1 > 93% of the predicted value. This seems to be the explanation for the absence of improvement in subjective aspects after addition of salmeterol to maintenance therapy with ICS.

Some studies in adults (13,14) and in children (8) with asthma have suggested that regular treatment with long-acting $ß_2$-agonists may lead to tolerance to its protective effects against bronchoconstrictor stimuli, such as methacholine (8,13) and exercise (14). Patients in these studies did not use ICS. In our study we observed that the mean difference of PC_{20} between the group receiving salmeterol and the control group decreased over three consecutive time intervals during 16 wk of treatment. Although this may easily happen by chance (two sided $p = 0.25$), it is not inconsistent with the phenomenon of tolerance. A more formal analysis for testing the treatment by time interaction did not yield any significance. Hence, this study, like others (15), did not show tolerance to methacholine provocation. This study was not designed to study this phenomenon, neither by its measurement scheme nor by its sample size.

A small rebound effect in bronchial responsiveness has been observed after cessation of treatment with short-acting $ß_2$-agonists (16,17). Although the clinical relevance of this finding is dubious, it may be possible that long-term treatment with long-acting $ß_2$-agonists induces a more pronounced rebound effect. We did not observe a rebound effect at 12 and 20 h or 1 wk after cessation of salmeterol with regard to bronchodilation and bronchial responsiveness. This confirms the results of

others who did not observe a rebound increase in bronchial responsiveness after 5 d up to 14.5 d after withdrawal of salmeterol (8,13,15,30,31).
We conclude that 16 wk of treatment with salmeterol in asthmatic children treated with maintenance therapy with ICS leads to a sustained bronchodilator effect during the day and improved circadian variation in airway diameter. The initial significant decrease in bronchial responsiveness disappeared, however, after 1 wk. Salmeterol has no additive beneficial effect on bronchial responsiveness, as no significant difference in PC_{20} methacholine or reduction in circadian variation of PC_{20} methacholine was observed from 1 wk up to 16 wk of treatment. Finally, cessation of salmeterol after 4 mo of treatment does not lead to a rebound increase in bronchial responsiveness in asthmatic children treated with ICS.

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CHAPTER 8

CIRCADIAN RHYTHM IN INFLAMMATORY PARAMETERS IN HEALTHY AND ALLERGIC ASTHMATIC CHILDREN TREATED WITH INHALED CORTICOSTEROIDS; INFLUENCE OF LONG-TERM TREATMENT WITH SALMETEROL

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Abstract

Though nocturnal asthma is frequently present in asthmatic children despite treatment with inhaled corticosteroids (ICS), there is little known on the role of inflammation in this respect. Neither is known whether the long-acting ß$_2$-agonist salmeterol may improve nocturnal asthma by way of reducing inflammation. We investigated daytime and nighttime inflammatory parameters in healthy children and compared baseline values with allergic asthmatic children already treated with ICS. Moreover, we assessed whether differences in inflammation between healthy and asthmatic children are associated with lung function parameters and whether long-term treatment with salmeterol influenced inflammatory parameters in asthmatic children. Ten healthy and 40 asthmatic children using ICS participated (aged 7 to 15 yrs). At baseline a blood sample was taken at 4:00 p.m. and 4:00 a.m. to analyze total number of leucocytes, lymphocytes, eosinophils, ECP, EDN, and lymphocyte subsets and activation markers (CD3, CD4, CD8, CD4/CD25, CD4/45Ro, CD4/HLAdr and CD8/CD28). At 4:00 p.m. and 4:00 a.m. asthmatic children performed a forced expiratory volume in one second (FEV_1); the day before baseline a 24 h peak expiratory flow (PEF) amplitude was measured (highest minus lowest value expressed as a percentage of the day's mean value). Asthmatic children enrolled in a randomized, double-blind, parallel study, receiving either twice daily 50 µg salmeterol xinafoate or placebo during 16 wk. At 4:00 p.m., asthmatic children had significantly higher values of leucocytes, lymphocytes, eosinophils, ECP, EDN, CD3+, CD4+, CD8+, CD4/CD45Ro+, and CD4/HLAdr+ cells than healthy children, this was also true for 4:00 a.m. values of ECP and EDN. ECP and EDN at 4:00 p.m. and 4:00 a.m. correlated significantly and positively with PEF amplitude (rho > 0.32; $p < 0.05$). At daytime CD3+, CD4+, CD8+, and CD45Ro+ correlated significantly and positively with FEV_1 % predicted (rho > 0.27; $p < 0.05$). Long-term treatment of salmeterol did not normalize inflammatory parameters. In conclusion: despite treatment with ICS, asthmatic children do have ongoing eosinophilic activation at day and night, and lymphocytic activation in daytime. Whereas a higher eosinophilic activation was related with a larger PEF variability, lymphocytic activation was not. The latter was, however, moderately associated with the level of airways obstruction (FEV_1). Salmeterol did not significantly improve inflammatory parameters in peripheral blood in allergic asthmatic children treated with ICS.

Introduction

Asthma is characterized pathologically by the presence of inflammation in the bronchial mucosa, with an influx and activation of eosinophils, lymphocytes and mastcells (1). Current guidelines recommend to institute regular use of anti-

inflammatory drugs in asthma when daily symptoms occur in order to suppress the inflammatory process, whereas monotherapy with short-acting or long-acting ß$_2$-agonists are regarded to be unsafe. Systemic side effects in the long-term management of the disease are usually observed only when daily doses of > 800 µg of inhaled corticosteroids (ICS) are used (2). Notwithstanding their proven effectiveness, clinical practice suggests that some children remain symptomatic despite the use of ICS (3,4). Therefore supplemental therapy may be necessary.

In severe symptomatic asthma an enhanced circadian variation in peak expiratory flow (PEF) is generally present, which has been associated with an increase in cell number and activation (5). A study in asthmatic adults showed that additional use of salmeterol provided a better symptomatic and lung functional benefit than doubling the dose of ICS (6). This beneficial effect may be largely due to the bronchodilator properties of the long-acting ß$_2$-agonist, yet some *in vitro* and *in vivo* studies suggest that an anti-inflammatory effect may play a role as well. For instance Di Lorenzo and coworkers (7) found a reduction of serum eosinophilic cationic protein (ECP) after 1 wk of treatment with salmeterol in mild asthmatics, as has been shown to occur with ICS as well (8). However, Gardiner and coworkers (9) could not show that 8 wk of treatment with salmeterol improved inflammatory parameters like T-cell activation and tryptase in bronchoalveolar lavage of asthmatics individuals who were already treated with ICS. However, this may well be due to the fact that healthy and asthmatic individuals had comparable values, thus improvement with salmeterol might not have been expected to occur. The study found a significant difference in the number of eosinophils and epithelial cells between healthy and asthmatic individuals, but again no effect of salmeterol was found. So far conflicting results with respect to anti-inflammatory properties of salmeterol have been presented.

Clinical effects of drugs are usually assessed by measuring symptoms, PEF (variability), forced expiratory volume in one second (FEV_1) and bronchial responsiveness. Improvement in these indices may, however, reflect different routes by which the improvement occurs. For instance the level of FEV_1 is determined by airway wall oedema, subepithelial fibrosis, and smooth muscle contraction, and hence both acute and chronic processes in the airway wall. In contrast, PEF variability (and not PEF level) may be considered to reflect to a large extent acute inflammatory changes in the airway wall. We therefore set out to assess the relationship between inflammatory parameters and these worldwide used indices of clinical outcome.We have been investigating allergic asthmatic children treated with ICS. A sustained bronchodilator effect and a reduced circadian variation in lung function occurred in these children after 16 wk of treatment with salmeterol (50µg twice daily) when compared with placebo. Addition of salmeterol did not lead to a clinically relevant supplementary gain with regard to bronchial responsiveness (10). In this paper we present the results of the same study with regard to daytime and nighttime changes in inflammatory parameters. We

compared our baseline values with results in healthy children. Finally we investigated whether the observed differences in inflammation between healthy and asthmatic children were associated with abnormalities in spirometry, PEF variability and bronchial responsiveness. We specifically focused on changes of eosinophils and lymphocytes.

Patients and methods

Subjects

Ten healthy controls (3 boys, 7 girls), aged 7 to 15 yrs participated. The healthy volunteers had no history of allergic and respiratory disease. They were characterized by total serum IgE in the normal range (11) and specific IgE to one or more common inhalation allergens (RAST class < 2, Pharmacia Diagnostics, Uppsala, Sweden), FEV_1 of at least 90% of the predicted value, and no increased bronchial responsiveness (histamine provocation concentration > 8 mg/ml [2 min tidal breathing, 5-min interval] causing a fall of 20% or more in FEV_1 from baseline). Forty children with a history of allergic asthma (23 boys, 17 girls), aged 7 to 15 yrs, participated. The asthmatic children were selected from our outpatient department. All were characterized by an increased total serum IgE and specific IgE to one or more common inhalation allergens (RAST ≥ 2, Pharmacia Diagnostics), FEV_1 of at least 70% of the predicted value, and increased bronchial responsiveness (histamine provocation concentration < 8 mg/ml [2 min tidal breathing inhalation, 5-min interval] causing a fall of 20% or more in FEV_1 from baseline). Symptoms were well-controlled for at least 4 mo before the study with maintenance ICS (twice daily 200 or 400 µg beclomethasone dipropionate rotadisk) and $ß_2$-adrenergic drug if needed (salbutamol rotadisk). None of the children used long-acting $ß_2$-adrenergic drugs before the start of the study.

Informed consent from all children and their parents was obtained. The study was approved by the Medical Ethics Committee of the University Hospital of Groningen.

Study Design

There was a baseline period (screening and baseline day) for all participating children. At the baseline day a blood sample was taken from all children at daytime (4:00 p.m.) and nighttime (4:00 a.m.) to collect inflammatory parameters in peripheral blood. The healthy children performed spirometry at daytime and the asthmatic children performed spirometry and a methacholine challenge test at daytime and at nighttime. The circadian PEF amplitude (highest minus lowest PEF value expressed as percentage of the day's mean value) was obtained at home by all subjects before the baseline day. After the baseline characterization, asthmatic children enrolled the long-term intervention study which had a randomized, double blind, placebo controlled, parallel design which was published earlier (10). They

were randomly assigned to one of the two groups, using either a dry powder inhaler (diskhaler) salmeterol xinafoate, 50 µg per inhalation (ICS + SLM group), or a matched placebo (ICS + PL group) at 8:00 a.m. and 8:00 p.m. First and last inhalations (after 16 wk of treatment) of the drug were supervised. During the long-term intervention there were regular clinical visits and telephone contacts for control of their asthma and adverse events (at 1, 4, 8, and 12 wk). After 16 wk of intervention (post-treatment period) a blood sample was taken from the asthmatic children and they performed spirometry and a methacholine challenge test at daytime and nighttime as well. The PEF amplitude was obtained at home one day before the hospital visit.

All children remained in hospital during the nocturnal measurements. Compliance of the study medication was checked after the study by counting the returned powder disks. The asthmatic children were allowed to use a dry powder inhaler (diskhaler) delivering salbutamol with a maximum of 1,600 µg daily as rescue medication. Rescue medication was stopped for at least 8 h before the measurements.

Processing of Blood

Total IgE and specific IgE were quantified using an enzyme immunoassay procedure (Pharmacia Diagnostics), and expressed in international units (IU)/ml, and Phadebas RAST units (PRU)/ml, respectively. Total leucocyte numbers in EDTA-anticoagulated blood were counted in a Coulter Counter (Model S-plus VI; Coulter electronics, Hialeah, USA). Viability was assesses by cellular exclusion of trypan blue. Cell differentiation was assessed on two slides after staining of blood smears with May-Grünwald-Giemsa. The average of 200 cell counts was taken for analysis.

ECP and eosinophil derived neurotoxin (EDN) were measured in serum obtained after coagulation of the blood for 1 h at room temperature. The supernatant was centrifuged twice for 10 min at 2000 g and stored at -80°C until analysis. Determination was performed by double antibody radioimmunoassays (Pharmacia Diagnostics AB, Uppsala, Sweden), as described previously (12,13). The detection level in both assays was 0.1 ng/ml.

Lymphocytes Markers

Lymphocyte subsets and activation state were analyzed by flowcytometry using double staining procedures, with a Facs 440 (Becton-Dickinson, Mountain View, CA, USA) equipped with an Argon laser and interfaced with a Hewlett-Packard Consort 30 computer as published previously (5). Cells were incubated with appropriate dilutions of mouse monoclonal antibodies (moabs, Becton-Dickinson, Mountain View, CA, USA) conjugated with a fluorochrome (fluorescein isothiocyanate [FITC] or phycoerythrin [PE] against CD3 (Leu-4, T-cells), CD4 (Leu 3a, T-helper/inducer cells), CD8 (Leu-2a, T-suppressor/cytotoxic cells). CD25

(α-chain interleukin 2 receptor), HLA-dr and UCHL (CD45Ro) were used in combination with anti-CD4 as indices of $CD4^+$ lymphocyte activation. CD28 (adhesion antigen presenting cell) was determed on $CD8^+$ cells. FITC and PE conjugated mouse immunoglobulins of matched isotypes (IgG_1, IgG_{2a}) were used as negative controls. The percentage of positive cells expressing the surface marker was assessed.

Blood lymphocytes and monocytes were separately gated according to their forward and sideward scatter, after verifying the purity using CD45 (Hle-1) and CD14 (Leu-m3) antibodies. EDTA-blood (100 µl) was fixed with 0.05% formaldehyde for 10 min at 22°C and centrifugated at 900 g for 2 min, then incubated for 15 min at 22°C with the appropriate dilutions of moabs supplemented with 10% AB serum. After lysis of red blood cells with lysing solution (155 mmol/l NH_4CL, 10 mmol/l KCl, NaAz 0.02%) the cells were washed, resuspended with PBS/BSA 0.5% and allowed to regenerate during 30 min at 22°C. A minimum of 20,000 events were collected for each sample. Analysis was performed using software (Lysis; Becton-Dickinson).

PEF Amplitude, FEV_1 and Methacholine Responsiveness

Short-acting $ß_2$-adrenergic drugs were withheld 8 h before the measurements. PEF measurements were performed every 4 h during 24 h in an upright position with a mini-Wright peak flow meter to calculate the circadian PEF amplitude. The best of three efforts was used for statistical analysis. FEV_1 was measured with a water-sealed spirometer (Lode BV, Groningen, the Netherlands). At least three reproducible values (i.e. < 5% difference between the recordings) were obtained; the highest was used for analysis.

Methacholine challenge tests were performed with a gauged DeVilbiss 646 nebulizer (DeVilbiss, Somerset, MA, USA), with an output of 0.13 ml/min according to the modified method of Cockcroft and coworkers (14). A 0.9% phosphate-buffered saline solution and doubling methacholine-bromide concentrations ranging from 0.038 to 19.6 mg/ml (equipotent to 0.03 to 16 mg/ml methacholine chloride) were inhaled for 2 min, with the nose clipped, at 5-min intervals, until FEV_1 had fallen by at least 20% from baseline FEV_1. The exact provocation concentration of methacholine that induced a 20% fall in FEV_1 (PC_{20}) was assessed by linear interpolation of the last two points of the log concentration response curve.

Data Analysis

Lymphocyte cell subsets are expressed as absolute number obtained by multiplying the percentage of positive cells with the total number of lymphocytes. Circadian variation was defined as daytime value minus nighttime value. FEV_1 values were expressed as percentage of the predicted value (% pred) (15). PC_{20} values were used after logarithmic transformation (base 2), since these reflect doubling doses

and have a Gaussian-shaped distribution. In subjects who did not reach a 20% fall in FEV_1 after the maximum dose of 19.6 mg/ml methacholine (baseline nighttime n =2 , posttreatment daytime n =2 , nighttime n = 2) PC_{20} was considered to be one doubling doses higher (39.2 mg/ml).
Distributions of variables with a Gaussian-shaped distribution were summarized by means and standard deviations (SD). For variables with a non-Gaussian-shaped distribution the median and range (minimum, maximum) were used. Groups were compared by using the unpaired *t* test (Gaussian-shaped variables), the Mann-Whitney *U* test (non-Gaussian-shaped or ordinal variables). Repeated measures analysis of variance (rmANOVA) was performed to compare the rhythm of the PEF values. Spearman's rho was obtained for correlations between lung function and inflammatory parameters. This was only performed if parameters differed significantly between healthy and asthmatic individuals. A p value less than 0.05 was considered to denote statistical significance. RmANOVA was performed with BMDP (module 5V), statistical package. All other analyses were performed with the SPSS/PC+ statistical software package.

Results

Baseline Period
One asthmatic subject in the placebo group did not complete the study because of reasons unrelated to the study, and was included in the analysis at the baseline-period.
The mean duration of having asthma was 8.3 yrs (SD 2.7 yrs). Baseline characteristics of the healthy and asthmatic children are shown in Table 1. Total IgE was significantly lower in the healthy group compared to the asthmatic children. PEF amplitude in the healthy subjects was significantly lower than in the asthmatic subjects, but the rhythm during 24 h was comparable (p = 0.33). FEV_1 values were not significantly different between the two groups. Daytime FEV_1 values of the asthmatic children were significantly higher than at nighttime (Table 1), this was not found for PC_{20} values. Daytime values of FEV_1 and PC_{20} were higher, but not significantly different in those who received 200 µg versus those who received 400 µg beclomethasone twice daily (FEV_1: 95.3 (13.1)% versus 88.4 (17.6)%, p = 0.21; geometric mean PC_{20} 1.2 mg/ml versus 0.5 mg/ml, p = 0.18).
Baseline characteristics of the asthmatic children and daily dosages of ICS were well in balance between the salmeterol and placebo groups (Table 1). Compliance was comparable between both treatment groups.

Healthy Versus Asthmatic Children
At daytime, healthy children had significantly lower values in the number of

leucocytes, lymphocytes, eosinophils, CD3+, CD4+, CD8+, CD45Ro+, and CD4/HLAdr+ lymphocytes compared to asthmatics. The same was true for serum ECP and EDN levels (Table 2 and 3).
At nighttime, healthy children had significantly lower numbers of leucocytes, lymphocytes, and eosinophils, as well as lower ECP and EDN levels than asthmatic children (Table 2). Lymphocyte subsets were not significantly different in healthy and asthmatic children (Table 3).
Healthy as well as asthmatic children had a significantly lower number of total lymphocytes and EDN at daytime than at nighttime. Total number of eosinophils was significantly lower at daytime than at nighttime in healthy children, but not in asthmatic children (Table 2). Healthy and asthmatic children had significantly lower number of CD3+, CD4+, CD8+, CD4/CD25+, CD45Ro+, and CD8/CD28+ cells in daytime compared with nighttime values. For CD4/HLAdr+ cells this was only found in healthy children (Table 3). Circadian variations in numbers of leucocytes, lymphocytes and their subsets, and eosinophils, and the levels of ECP and EDN were not significantly different between the healthy and the asthmatic children.

Relationship of Inflammatory Parameters with Lung Function in Asthmatic Children

ECP and EDN correlated significantly and positively with PEF amplitude at daytime and nighttime (rho > 0.32; $p < 0.05$). At daytime, CD3+, CD4+, CD8+, and CD45Ro+ cells correlated significantly and positively with FEV_1 % pred (rho > 0.27; $p < 0.05$). None of the inflammatory parameters correlated with the circadian variation in FEV_1. At daytime, the total number of CD4/HLAdr+ cells, and at nighttime the total number of lymphocytes correlated significantly and positively with PC_{20} (rho > 0.28; $p< 0.05$). See Table 4.

Changes after Treatment with Salmeterol

The change in circadian variation with 16 wk of treatment of salmeterol compared to placebo was not significantly different for all inflammatory parameters measured. This was also true for absolute daytime values (Table 5 and 6).
At night, we observed significant differences due to treatment between the group that received salmeterol and the placebo group with regard to changes in the number of lymphocytes, CD8+ and CD8/CD28+ cells (number of lymphocytes in the ICS + SLM group: -0.3 [-1.5 - 0.6] versus ICS + PL group: 0.1 [-1.3 - 1.5], $p = 0.04$; number of CD8+ cells in the ICS + SLM group: -0.1 [-0.5 - 0.2] versus ICS + PL group: 0.0 [-0.3 - 0.4], $p = 0.03$; number of CD8/CD28+ cells in the ICS + SLM group: -0.1 [-0.3 - 0.2] versus ICS + PL group: 0.0 [-0.2 - 0.5], $p = 0.05$). See Table 5 and 6.

TABLE 1 Baseline characteristics of the subjects by group

	Healthy	Asthma	Baseline of study	
		total group	ICS + SLM	ICS + PLA
	n = 10	n = 40	n = 20	n = 20
Age, yrs	12.6 ± 2.1	11.4 ± 2.6	11.4 ± 2.4	11.4 ± 2.8
ICS 400/800	-	31/9	13/7	18/2
Total IgE, IU/ml	14 (2 - 180)**	573 (12 - 2000)	557 (94 - 2000)	585 (12 - 2000)
PEF amplitude, %	9.7 (5.1 - 11.9)*	14.2 (4.2 - 71.3)	14.6 (4.5 - 69.1)	13.9 (4.2 - 71.4)
Daytime				
FEV_1,% pred	95.3 ± 5.9	93.7 ± 14.3#	93.6 ± 12.9	93.8 ± 15.9
PC_{20} methacholine Geom. mean (range), mg/ml	not done	1.0 (0.04 - 9.43)	1.20 (0.04 - 7.44)	0.84 (0.04 - 9.43)
Nighttime				
FEV_1, % pred	not done	90.3 ± 16.0	91.6 ± 13.5	89.0 ± 18.4
PC_{20} methacholine geom. mean (range), mg/ml	not done	1.2 (0.05 - 39.2)	1.41 (0.09 - 39.2)	1.04 (0.05 - 10.4)

Values are expressed as mean (standard deviation) or median (minimum - maximum) depending on the skewness of the distribution. ICS: inhaled corticosteroids, 400/800: number receiving 200 µg or 400 µg beclomethasone twice daily, pred: predicted, geom. mean: geometric mean, SLM: salmeterol, PLA: placebo. Statistical analysis is performed by Student's *t* tests or Mann-Whitney *U* tests depending of the skewness of the distribution. *: $p < 0.05$, **: $p < 0.001$ for healthy versus asthmatic children. #: $p < 0.05$ daytime value versus nighttime value.

TABLE 2 Inflammatory parameters in peripheral blood at daytime and nighttime in healthy (n = 10) and in asthmatic (n = 40) children

	Healthy	Asthma
Daytime		
Leucocytes, 10^9/l	5.5 (3.2 - 7.5)**	7.8 (4.7 - 14.2)
Lymphocytes, 10^9/l	1.8 (1.5 - 3.3)*,#	2.8 (0.9 - 6.2)##
Eosinophils, 10^6/l	115 (55 - 470)**,#	471 (77 - 1,820)
ECP, ng/ml	7.5 (2.3 - 14.5)*	12.1 (4.2 - 27.9)
EDN, ng/ml	15.6 (11.2 - 24.3)**,#	31.8 (7.2 - 75.6)##
Nighttime		
Leucocytes, 10^9/l	6.2 (3.9 - 7.5)**	7.7 (5.0 - 13.1)
Lymphocytes, 10^9/l	3.2 (1.2 - 4.8)*	3.4 (1.2 - 4.8)
Eosinophils, 10^6/l	200 (140 - 610)**	520 (143 - 1,350)
ECP, ng/ml	6.9 (3.1 - 15.2)*	12.5 (4.6 - 34.5)
EDN, ng/ml	19.9 (13.3 - 27.5)**	35.0 (11.5 - 101.5)

Values are expressed as median (minimum - range), statistics differences with Mann-Whitney *U* tests. *: $p < 0.05$, **: $p < 0.001$, healthy versus asthmatic children. #: $p < 0.05$, ##: $p < 0.001$, daytime versus nighttime value.

TABLE 3 Absolute number of lymphocyte subset positive cells (10^9/l) at daytime and nighttime in healthy (n = 10) and asthmatic (n = 40) children

	Healthy		Asthma
Daytime			
CD3	1.4 (1.1 - 1.9)	*	1.9 (0.6 - 4.7)
CD4	0.8 (0.6 - 1.2)	*	1.2 (0.3 - 2.8)
CD8	0.5 (0.3 - 1.0)	*	0.8 (0.3 - 1.7)
CD4/CD25	0.1 (0.0 - 0.1)		0.1 (0.0 - 0.1)
CD45Ro	0.3 (0.2 - 0.6)	*	0.4 (0.1 - 0.5)
CD4/HLAdr	0.0 (0.0 - 0.0)	*	0.0 (0.0 - 0.1)
CD8/CD28	0.4 (0.2 - 0.7)		0.5 (0.2 - 1.1)
Nighttime			
CD3	2.0 (1.5 - 3.0)#		2.5 (0.9 - 3.6)#
CD4	1.1 (0.8 - 1.9)#		1.4 (0.5 - 2.4)#
CD8	0.8 (0.6 - 1.1)#		1.0 (0.4 - 1.7)#
CD4/CD25	0.1 (0.1 - 0.2)#		0.1 (0.0 - 0.2)##
CD45Ro	0.3 (0.3 - 0.8)#		0.4 (0.1 - 0.7)#
CD4/HLAdr	0.1 (0.0 - 0.1)#		0.1 (0.0 - 0.1)
CD8/CD28	0.6 (0.4 - 1.0)#		0.7 (0.2 - 1.2)##

Values are expressed as median (minimum - range), statistics differences with Mann-Whitney *U* tests. *: $p < 0.05$ healthy versus asthmatic children. #: $p < 0.05$, ##: $p \leq 0.001$, daytime versus nighttime.

TABLE 4 Correlations (Spearman's rho) in asthmatic children (n = 40) between PEF amplitude, FEV_1 % pred, FEV_1 day-night variation and log_2PC_{20} with inflammatory parameters in peripheral blood. Only analysis on data significantly different between healthy and asthmatic children

	PEF amplitude	FEV_1, % pred	log_2 PC_{20}
Daytime			
Leucocytes, 10^9/l	0.21	0.22	-0.08
Lymphocytes, 10^9/l	0.15	0.18	0.22
Eosinophils, 10^6/l	0.22	0.19	0.08
ECP, ng/ml	0.35*	-0.01	-0.19
EDN, ng/ml	0.32*	-0.05	-0.18
CD3, 10^9/l	0.24	0.27*	0.24
CD4, 10^9/l	0.24	0.27*	0.25
CD8, 10^9/l	0.14	0.27*	0.23
CD45Ro, 10^9/l	0.18	0.38*	0.22
CD4/HLAdr, 10^9/l	0.04	0.11	0.34*
Nighttime			
Leucocytes, 10^9/l	0.22	-0.07	0.15
Lymphocytes, 10^9/l	0.25	-0.09	0.28*
Eosinophils, 10^6/l	0.13	0.03	0.10
ECP, ng/ml	0.32*	-0.08	0.01
EDN, ng/ml	0.38*	-0.01	-0.15

% pred = percentage predicted value. *: rho < 0.05.

TABLE 5 Inflammatory parameters in peripheral blood at nighttime in asthmatic children treated with inhaled corticosteroids (ICS) with salmeterol (SLM) (n = 20) or ICS with placebo (PLA) (n = 20) at pre-treatment and post-treatment period

	ICS + SLM		ICS + PLA	
	pre-treatment	post-treatment	pre-treatment	post-treatment
Daytime				
Leucocytes, 10^9/l	7.5 (5.4 - 13.2)	7.0 (4.2 - 9.9)	8.0 (4.7- 14.2)	8.0 (5.0 -14.2)
Lymphocytes, 10^9/l	2.8 (0.8 - 6.2)	2.6 (1.2 - 4.2)	2.8 (0.9 - 4.5)	2.9 (1.7 - 4.8)
Eosinophils, 10^6/l	445 (77 - 1820)	350 (160 - 610)	498 (190 - 1309)	410 (22 -1694)
ECP, ng/ml	10.2 (4.2 - 23.5)	11.1 (2.9 - 41.6)	13.9 (6.0 -27.9)	14.4(2.5- 38.4)
EDN, ng/ml	32.6 (10.5- 59.5)	28.2 (9.5- 114.4)	31.1 (7.2- 75.6)	32.7(9.4- 65.4)
Nighttime				
Leucocytes, 10^9/l	7.4 (5.0 - 10.4)	6.9 (3.5 - 11.3)	7.9 (6.2-13.1)	8.3 (5.0 -11.2)
Lymphocytes, 10^9/l	3.5 (1.2 - 5.0)	3.2 (1.4 - 5.1)	3.4 (2.1 - 4.6)	3.8 (2.0 - 5.0)*
Eosinophils, 10^6/l	478 (143 - 1350)	351 (100 - 640)	594 (220 - 1070)	485 (132-1925)
ECP, ng/ml	10.7 (5.3 - 28.4)	10.6 (3.5 - 40.6)	13.1 (4.6 - 34.5)	10 (4 - 38)
EDN, ng/ml	34.8 (11.5- 65.6)	30.7 (10.8-115.8)	36.3 (18.7-101.5)	38.3(13.1-67.2)

Values are expressed as median (range), statistic differences with Mann-Whitney *U* test.

*: $p < 0.05$ for changes due to treatment at night ICS + SLM versus ICS + PLA.

TABLE 6 Absolute number of lymphocyte subsets (10^9/l) at day and nighttime in asthmatic children treated with inhaled corticosteroids (ICS) and salmeterol (SLM) (n = 20) or ICS and placebo (PLA) (n = 20) at pre-treatment and post-treatment period

	ICS + SLM		**ICS + PLA**	
	pre-treatment	post-treatment	pre-treatment	post-treatment
Daytime				
CD3	2.0 (0.6 - 4.7)	1.8 (0.9 - 3.1)	1.9 (0.7 - 2.6)	1.9 (1.1 - 3.5)
CD4	1.2 (0.3 - 2.8)	1.1 (0.5 - 2.0)	1.2 (0.3 - 1.6)	1.2 (0.6 - 2.5)
CD8	0.8 (0.3 - 1.7)	0.8 (0.4 - 1.2)	0.7 (0.3 - 1.0)	0.8 (0.4 - 1.1)
CD4/CD25	0.1 (0.0 - 0.1)	0.1 (0.0 - 0.2)	0.1 (0.0 - 0.2)	0.1 (0.0 - 0.2)
CD45Ro	0.4 (0.1 - 0.5)	0.3 (0.1 - 0.6)	0.4 (0.2 - 0.5)	0.4 (0.1 - 0.5)
CD4/HLAdr	0.1 (0.0 - 0.1)	0.0 (0.0 - 0.1)	0.0 (0.0 - 0.1)	0.1 (0.0 - 0.1)
CD8/CD28	0.5 (0.2 - 1.1)	0.5 (0.3 - 1.0)	0.5 (0.2 - 0.7)	0.5 (0.3 - 0.7)
Nighttime				
CD3	2.5 (0.9 - 3.6)	2.2 (1.1 - 4.1)	2.5 (1.3 - 3.2)	2.7 (1.3 - 3.4)
CD4	1.3 (0.5 - 2.4)	1.3 (0.1 - 2.4)	1.4 (0.8 - 2.0)	1.7 (0.9 - 2.4)
CD8	1.0 (0.4 - 1.7)	0.9 (0.4 - 1.5)	0.9 (0.4 - 1.5)	1.1 (0.4 - 1.5)*
CD4/CD25	0.1 (0.0 - 0.2)	0.1 (0.1 - 0.3)	0.1 (0.0 - 0.2)	0.2 (0.1 - 0.3)
CD45Ro	0.4 (0.1 - 0.6)	0.4 (0.2 - 0.7)	0.4 (0.3 - 0.7)	0.5 (0.1 - 0.7)
CD4/HLAdr	0.1 (0.0 - 0.1)	0.0 (0.0 - 0.1)	0.1 (0.0 - 0.1)	0.1 (0.0 - 0.1)
CD8/CD28	0.7 (0.4 - 1.2)	0.7 (0.3 - 1.1)	0.7 (0.2 - 1.1)	0.7 (0.3 - 1.1)*

Values expressed as median (minimum - maximum), statistic differences with Mann-Whitney *U* test. *: $p < 0.05$ for changes due to treatment at night ICS + SLM versus ICS + PLA.

Discussion

This study shows that asthmatic children with high doses of inhaled corticosteroids (ICS) still have ongoing inflammation, since we found higher serum ECP and EDN levels both day and night as well as higher numbers of peripheral blood lymphocytes and especially activated CD4+ lymphocytes in daytime than in healthy children. PEF variability was positively and significantly related with higher ECP and EDN levels both in daytime and nighttime, whereas FEV_1 was significantly associated with activated lymphocytes in daytime, as was bronchial responsiveness. Finally, 16 wk of salmeterol treatment did not change the above mentioned inflammatory parameters to the normal range.

The present study is the first of which we are aware that documents circadian inflammatory parameters in peripheral blood in asthmatic children. There are only a few studies on inflammatory parameters in peripheral blood in children. Three weeks after allergen avoidance in a high altitude climate, a significant reduction in CD25+/CD4+ cells in peripheral blood of asthmatic children was found, combined with a reduction in the number of eosinophils (16). Gemou and coworkers (17) who compared the absolute numbers of CD4+ and CD8+ T-cells in peripheral blood between asthmatic allergic children and non-asthmatic-allergic children and found no significant difference between both groups, whereas the absolute number of eosinophils of asthmatic children was elevated compared to the non-asthmatic children. They did not compare their data with healthy children. We found a difference in inflammatory parameters in peripheral blood between healthy and asthmatic children treated with ICS. Variations in T-lymphocytes in asthmatic patients are related to the disease severity and may therefore differ from healthy subjects. One study showed the numbers of activated T-cells to be related to the severity of asthma, as measured by impairment of FEV_1, and increased methacholine bronchial responsiveness (18). This is in contrast with Gerblich and coworkers who concluded that T-cell subsets in peripheral blood of asymptomatic adult asthmatics did not differ from healthy subjects (19). Since our patients were still symptomatic and mildly hyperresponsive and furthermore were much younger in age, this might explain the different findings. All these studies were performed at daytime. Although we found some differences in inflammatory parameters between daytime and nighttime values of asthmatic versus healthy children, the day-night variation was not different. This may be due to treatment with ICS.

A drawback of our study is that we assessed our inflammatory parameters in peripheral blood. Peripheral blood contains only 2% of all lymphocytes and may not contain highly differentiated antigen-specific T-cells, which are specifically localized in inflammatory tissues. It thus only partly reflects the inflammatory process in the airway walls. However, bronchoalveolar lavage in children is not easily done, nor acceptable for the child without sedation. We therefore first started to look at peripheral blood inflammatory parameters. Lymphocytes are recruited into peripheral blood under allergen challenge conditions in sensitized asthmatic children (20). Virchow and coworkers (21) found that after segmental allergen challenge the number of neutrophils and activated CD4+ cells (CD25+) increased significantly in peripheral blood, but no change in eosinophils and other leucocytes or lymphocyte subsets. This type of challenge does, however, reflect an acute event after a supraphysiologic dose of allergen and may not represent the ongoing

chronic inflammatory process we have been investigating in our children. A study of Oosterhoff and coworkers (5), containing patients with increased circadian PEF variability, found that a higher number of HLA-dr/CD4+ cells in lavage fluid was associated with increasing circadian PEF rhythm, but this was not present with peripheral blood values. This finding is compatible with our observations.

We assessed associations between the inflammatory parameters that were significantly different between healthy and asthmatic children and the critical outcome measures in clinical studies, i.e. FEV_1, PEF amplitude and bronchial responsiveness. We found, although with a rather small rho value (explaining about 10% of the variation), that a larger PEF variability was related to higher level of eosinophilic activation. The level of FEV_1, and to a smaller extent bronchial responsiveness was, however, not associated with eosinophilic activation but with lymphocyte activation. The interpretation of the findings is difficult given their finding in peripheral blood (see above). It is attractive to speculate that acute eosinophilic activation (both day and night) is important to the acute variability in airway diameter, i.e. PEF variability, whereas this has not such a large effect on the level of fixed airway obstruction. This increased eosinophilic activation has already been found to occur 3 h after allergen challenge (22). In contrast, the reduction in the absolute level of FEV_1 is more likely due to both acute and chronic ongoing inflammatory processes in the airway wall. A higher activation of peripheral blood lymphocytes has been previously found after the late allergic reaction upon allergen challenge (21), supporting the slower onset of lymphocyte activation than eosinophilic activation. We observed a higher FEV_1 to be associated with higher chronic lymphocyte activation, as evidenced by increased expression of CD45Ro, combined with a somewhat more acute activation parameter HLA-dr. We would have expected a negative correlation if the hypothesis that chronic activation leads to irreversible damage. However, it has been mentioned before that lymphocyte activation does not concur between different compartments, such as peripheral blood, bronchalveolar lavage fluid and airway wall tissue (23,24). Thus, biopsy studies have to show whether the number of CD4/CD45Ro+ cells are higher in airway wall biopsies of asthmatic individuals with lower FEV_1 values.

ß-Agonists have effects on a wide variety of cell types in the airways and on inflammatory cells. Little is known about the effect *in vivo* of salmeterol on inflammatory parameters. Gardiner and coworkers (9) studied in asthmatic adults receiving regular ICS therapy the effect of salmeterol on lavage-indices, but did not find a significant change in percentage CD4+, CD8+ or proportion of HLA-dr expressing lymphocytes after 8 wk of salmeterol treatment. Taylor and coworkers (25) concluded that salmeterol and salbutamol did not inhibit mediator release from pulmonary inflammatory cells, whereas Baker and coworkers (26) found that salmeterol inhibits the release of thromboxane B2 from both airway macrophages and freshly isolated blood monocytes *in vitro*. ß-Agonists have little or no effect on the chronic inflammatory response that underlies airway hyperresponsiveness and chronic asthma. This is most clearly demonstrated by biopsy studies showing that regular treatment with ß-agonists, including salmeterol, fails to resolve the inflammatory process, as judged by inflammatory indices in bronchial biopsies or bronchoalveolar lavage (9,27,28). We found a significant reduction of nighttime total lymphocytes as also found with short-acting $ß_2$-agonists (29) during salmeterol treatment, but the clinical relevance is unknown. Our data support previous findings in that inhaled

ß$_2$-agonists do not suppress the underlying inflammation of asthma in the way steroids do, and yet they may control the symptoms of asthma.
In summary our data show that asthmatic children, despite their treatment with ICS, do have ongoing eosinophilic activation at day and night and lymphocytic activation in daytime. Eosinophilic activation was moderately related to the variability in airway diameter, whereas this was not the case for lymphocytic activation. Despite clinical improvement with salmeterol treatment, we could not find any improvement in inflammatory parameters in daytime, even though there was room for improvement given the observed differences in inflammatory parameters between healthy and asthmatic children treated with ICS.

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CHAPTER 9

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Nocturnal respiratory symptoms are common in children with asthma. This is generally attributed to increased airway obstruction and hyperresponsiveness at night. A circadian variation in airway diameter has been described both in healthy children and in children with asthma. In healthy children this 24 hours variation is small, and it is enhanced in many asthmatic patients, especially when in a unstable phase of the disease. The best lung function values occur during the daytime, while trough values are generally measured at the end of the night. This causes patients and their parents to wake up with negative consequences for school performance and disruption of family life.

Nocturnal complaints of asthma have been recognized for a long time. An increased nocturnal airflow limitation has already been described in the fourth century AD. In the forties and fifties of this century, research into determinants of increased airway obstruction began, to which the Groningen group contributed. Up to now, research on the phenomenon of nocturnal airflow limitation has largely focussed on endogenous rhythms, such as the contribution of variations of the autonomic nervous system, variations in cortisol secretion, or associations of variations in inflammatory variables and the nocturnal fall in lung function. Although parts of the puzzle have been elucidated, other parts are still unknown. During earlier studies in allergic asthmatic children we have observed that the nocturnal airflow limitation as measured at home improved during a short stay in hospital. This led to the hypothesis that next to endogenous factors, exogenous (environmental) triggers such as allergens and tobacco smoke were also able to modulate the circadian variation in airflow limitation.

This thesis aimed to give more insight in, on one hand, the epidemiology of nocturnal respiratory symptoms in children with asthma who are regularly controlled on an outpatient clinic for asthmatic children. On the other hand, it tries to answer the question whether environmental triggers are of importance for the magnitude of the circadian variation in airflow limitation. As it has been suggested that the inflammatory process in the lungs is more active during the night, environmental triggers, such as inhaled allergens, could activate this inflammatory process. Several studies suggested that the inflammatory process is more severe during the night in patients with nocturnal airflow limitation. These studies show an increase of inflammatory cells and mediators in blood and urine during the night (1-4). More direct evidence is shown by an increase in inflammatory cells and activation markers in bronchoalveolar lavage fluid obtained during the night (5,6). Anti-inflammatory drugs such as inhaled corticosteroids (ICS) are known to reduce the degree of inflammation and reduce 24 hours variation in peak expiratory flow (PEF) (7,8).

Long-acting $ß_2$-agonists have proven to be especially beneficial to overcome the nocturnal fall in lung function in asthmatic patients (9). In the earliest studies it has been suggested that they possessed also anti-inflammatory properties, since a single inhalation prevented not only the early asthmatic reaction after allergen inhalation,

but also the late asthmatic reaction (10). Therefore, we studied the effect of 16 weeks treatment with the long-acting ß$_2$-agonist salmeterol on daytime and nighttime lung function, bronchial responsiveness and inflammatory variables in peripheral blood of allergic children treated with ICS.

Chapter 1.1 contains a literature study on the epidemiology of nocturnal symptoms of asthma in different populations. Furthermore, the literature on mechanisms that may modulate the circadian variation in lung function is discussed. It is generally accepted that inflammation of the airways underlies the pathogenesis of asthma. The 24 hours variation in endogenous rhythms such as bronchial responsiveness, the autonomic central nervous system and cortisol secretion modulate the inflammatory processes in the airways, resulting in variation of the airway diameter over 24 hours. In our concept of nocturnal airflow limitation in asthmatic children we hypothesize that the severity of this basic inflammatory process may be enhanced by exogenous triggers, such as exposure to allergens and non-allergic triggers, resulting in larger circadian swings in lung function.

In *Chapter 2* we describe a study in which we investigated the frequency of nocturnal symptoms such as cough, wheeze, shortness of breath, and dyspnea on awakening in the morning in 796 consecutive children with asthma attending our outpatient clinic. Nocturnal symptoms were reported in 47% of the children; 6% every night and 34% at least once a week. Only 38% of these 47% with nocturnal symptoms reported these spontaneously. Patients with nocturnal symptoms had a lower FEV_1, perceived their asthma as more severe, and had their daytime activities more affected than those without nocturnal symptoms. FEV_1 seemed to be a poor predictor for nocturnal symptoms. These results confirm that nocturnal symptoms of asthma reflect a more severe disease state. Furthermore it shows that doctors should specifically ask about nocturnal symptoms as they are not spontaneously mentioned. This offers the possibility to introduce appropriate treatment.

In *Chapter 3* we have tried to answer the question whether house dust mite (HDM) exposure levels in living and bedrooms of 25 asthmatic children are higher than in those of age and sex matched healthy children, living in the same area. HDM allergen (HDMA) concentrations were not significantly different between the two groups, although a higher cleaning frequency, and more smooth floor coverings were reported in the asthmatic group. We observed that low HDMA concentrations were a general finding in Dutch dwellings in the present generation of children. Smooth floor coverings contained less fine dust and lower concentrations of HDMA than carpeted floors. The large interindividual variation in HDMA concentrations in the different houses suggests an individual approach with regard to environmental measures.

Chapter 4 contains a study in which we investigated in 55 asthmatic children with a mono-allergy to HDM the contribution of exogenous triggers, such as environmental tobacco smoke, the presence of pets, and levels of HDMA in living rooms, bedrooms, mattresses (n=25) and classrooms to an increased circadian PEF amplitude (PEF value every 4 hours during 24 hours expressed as highest minus lowest value expressed as a percentage of the day's mean value). All children were well controlled with daily ICS. To investigate the influence of these triggers on the circadian PEF amplitude, ICS were withdrawn for 6 days. We found that exposure to environmental tobacco smoke, the presence of pets and high exposure to HDMA concentrations in bedding contributed independently to a higher PEF amplitude after withdrawal of ICS. Highest HDMA exposure sources were mattresses and carpets on a smooth floor.

In *Chapter 5* a study is presented assessing whether a seasonal difference in HDMA exposure contributed to an increase in circadian PEF amplitude in 25 asthmatic children with a mono-allergy to HDM. In all children HDMA were collected in living rooms, bedrooms and from the surface of mattresses by vacuum cleaning. In both spring and autumn, PEF amplitude was measured before and 6 days after withdrawal of ICS. This cross- sectional study showed that a higher PEF amplitude was not significantly associated with higher HDMA exposure in mattresses. However, the change in HDMA exposure over seasons (autumn value minus spring value) contributed significantly to the change in PEF amplitude after withdrawal of ICS.
The results of both studies in chapter 4 and 5 strongly suggest that superimposed exogenous triggers enhance the magnitude of the circadian variation in airway diameter next to endogenous modulation.

Chapter 6 is a *letter to the editor* in answer to a study of De Lovinfosse *et al.* published in *Allergy* 1994; 49: 64-66 in which the authors showed a correlation between HDM specific IgE and HDMA exposure levels and suggested that mite specific IgE could be used as a surrogate for mite exposure. In 25 asthmatic children with an isolated allergy to HDM we tried to correlate the same variables (serum mite specific IgE and HDMA levels collected from mattresses). We did not observe a correlation and we could not confirm their statement. This seems logical since the immunological ability of an individual to react to a certain amount of HDM is a probably more relevant factor that may influence IgE production.

In *Chapter 7* we present results of a study in forty asthmatic children who were already on daily ICS and randomly treated for 16 weeks with the long-acting $ß_2$-agonist salmeterol or placebo. The effects on FEV_1 and bronchial responsiveness both during the day and overnight were investigated. Furthermore, we assessed whether cessation of salmeterol after 4 months led to a rebound increase in

bronchial responsiveness. We observed in the salmeterol group a sustained higher FEV_1 and an improved circadian variation in airway diameter than in the placebo group from 1 to 16 weeks of treatment. Overall mean PC_{20} methacholine from 1 to 16 weeks of treatment was not significantly different between the salmeterol and placebo groups. This lack of improvement in PC_{20} in the salmeterol group could not be explained by a ceiling effect since all children had moderate to severe bronchial responsiveness and all children had enough room for improvement. Cessation of salmeterol after 16 weeks of treatment did not lead to a rebound increase in bronchial responsiveness.

In *Chapter 8* we present another aspect of this same study and investigated whether addition of salmeterol to treatment with ICS leads to a beneficial effect on inflammatory variables in peripheral blood during the day as well as overnight. Blood was collected from the same children in the same study as mentioned in chapter 7. Besides this question, we investigated whether a difference in these inflammatory variables existed between the asthmatic children and a healthy control group, and whether these differences in inflammatory parameters are associated with lung function parameters. We observed that children, despite their treatment with ICS, do have ongoing eosinphilic activation at day and night and lymphocytic activation in daytime. Eosinophilic activation was related to the variability in airway diameter, whereas lymphocytic activation was associated with the level of FEV_1. Despite clinical improvement with salmeterol treatment, we could not find any improvement in inflammatory parameters in daytime, even though there was room for improvement given the observed differences in inflammatory parameters between healthy and asthmatic children treated with ICS.

Final conclusions and recommendations

Nocturnal symptoms are still frequently present in a population of children with asthma who are under control in an asthma outpatient clinic. FEV_1 seems to be a poor predictor for nocturnal symptoms, and doctors should specifically ask for these symptoms because this offers the opportunity to take adequate measures. Since the end of this epidemiological study on the frequency of nocturnal symptoms newer therapeutic options such as mattress encasings and drugs especially beneficial for nocturnal symptoms became available. It seems worthwhile to repeat such a study in the near future because this may give insight in possible shifts in nocturnal symptoms in this population.
The studies in this thesis add new insights to the concept on the pathophysiology of nocturnal airflow limitation in asthmatic children. Exogenous factors such as environmental tobacco smoke, the presence of pets, and high HDMA levels all independently contribute to the circadian PEF amplitude in allergic asthmatic

children. Parents should not only be stressed to stop smoking during pregnancy, but also any time thereafter to improve the stability and the prognosis of their childs asthma. Another important message is that pets contribute to an enhanced circadian PEF variability even in asthmatic children who do not express allergies to these pets. It seems appropriate to assess whether withdrawal of these pets improves asthma stability. HDM in mattresses provide the most important contribution to the circadian variation in airway diameter compared to other sources of HDM exposure.
Children with asthma are exposed to comparable HDM levels as healthy children. More smooth floor coverings were observed in asthmatic children probably as a result of earlier given advises. Smooth floors contained less HDM than carpeted floors, indicating that environmental advises should include the elimination of carpeted floors.
The concept of the circadian variation in airway diameter in asthmatic children that superimposed endogenous circadian rhythms such as for bronchial responsiveness, the autonomic central nervous system and cortisol secretion play an important and intricate role in the circadian modulation of the inflammatory process by changing numbers of cells, their release of mediators and/or the susceptibility of airway smooth muscle and vasculature needs further support. It is an attractive concept to hypothesize that endogenous secretion of cortisol conducts this inflammatory process. Low cortisol levels at night, or a more general lower cortisol secretion in asthmatic children with nocturnal symptoms oppose possible protection against inflammatory processes. This needs further investigation.
Long-acting ß$_2$-adrenergic bronchodilators are a good tool in the treatment of nocturnal airflow limitation. Even in stable asthmatic children who were already treated with ICS we observed a sustained bronchodilating effect of salmeterol and a reduction in circadian airflow limitation. We did not find a protective effect on bronchial responsiveness despite that all children had enough room to improve. We did not observe a rebound effect on bronchial responsiveness after cessation of salmeterol. Little evidence remains that salmeterol has anti-inflammatory properties.

Future studies on treatment of nocturnal symptoms in allergic asthmatic children should focus on optimal treatment regimes. Mattress encasings and long-acting ß$_2$-adrenergic drugs such as salmeterol seem to be successful interventions together with anti-inflammatory treatment with ICS. However, smoking cessation and pet avoidance should be advised in every child with unstable asthma.

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SAMENVATTING, CONCLUSIES EN AANBEVELINGEN

Nachtelijke luchtwegklachten worden vaak gemeld bij kinderen met astma. Over het algemeen wordt dit toegeschreven aan een toegenomen vernauwing en bronchiale hyperreaktiviteit (gevoeligheid/prikkelbaarheid van de luchtwegen) gedurende de nacht. Zowel bij gezonde kinderen als bij kinderen met astma is een circadiane (24 uurs) variatie in luchtwegdiameter beschreven. Deze 24 uurs variatie in luchtwegdiameter is bij gezonde kinderen klein. Bij mensen met astma kan deze variatie uitgesproken zijn, met name tijdens een instabiele periode van de ziekte. De beste longfunktiewaarden worden overdag gemeten, terwijl de slechtste waarden over het algemeen ’s nachts gemeten worden. Dit heeft tot gevolg dat kinderen met astma, en hun ouders, ’s nachts wakker worden met als gevolg slechtere schoolprestaties en verstoring van het gezinsleven.

Nachtelijke klachten ten gevolge van astma zijn al lange tijd onderkend en beschreven. De eerste meldingen van nachtelijk toegenomen luchtwegvernauwing stammen uit de vierde eeuw na Chr. In de veertiger en vijftiger jaren van deze eeuw begon de wetenschappelijke interesse naar de nachtelijk toegenomen luchtwegvernauwing te ontstaan. De Groninger onderzoeksgroep leverde een bijdrage hieraan. Tot nu toe heeft het wetenschappelijk onderzoek zich met name bezig gehouden met endogene ritmes, zoals de bijdrage van de variatie van het autonome zenuwstelsel, variaties in cortisol produktie, of de associatie met variatie in inflammatoire (ontstekings) parameters en de nachtelijke daling in longfunktie. Hoewel delen van de puzzel opgehelderd zijn, zijn andere gedeelten nog volstrekt onbekend. Tijdens een eerdere studie bij allergische kinderen met astma vonden we dat de nachtelijke luchtwegvernauwing die thuis was gemeten, significant verminderde tijdens een kort verblijf in het ziekenhuis. Hieruit ontstond de hypothese dat naast endogene factoren, ook exogene factoren (omgevingsfactoren) zoals allergenen en sigarettenrook een bijdrage leveren aan de circadiane variatie in luchtwegdiameter.
Dit proefschrift tracht meer inzicht te geven in de epidemiologie van nachtelijke luchtwegklachten bij astmatische kinderen die regelmatig een specialistische polikliniek voor astma bezoeken. Tevens wordt in dit proefschrift getracht de vraag te beantwoorden of omgevingsfactoren van invloed zijn op de mate van nachtelijke luchtwegvernauwing bij allergische kinderen met astma. Zoals gezegd wordt verondersteld dat het inflammatoire proces in de longen ’s nachts toeneemt. Omgevingsfactoren, zoals allergenen, zouden dit inflammatoire proces kunnen activeren.
Diverse onderzoeken suggereren dat het inflammatoire proces gedurende de nacht actiever is bij mensen met nachtelijk toegenomen luchtwegvernauwing. Deze studies laten een toename van ontstekingscellen en mediatoren in het bloed en in de urine zien bij patienten met nachtelijke luchtweg vernauwing, in tegenstelling tot patiënten die dit niet hebben (1-4). Meer directe bewijzen hiervoor zijn de toegenomen hoeveelheid ontstekingscellen en activatie van ’markers’ in bronchoalvelolaire spoelvloeistof (longspoeling) gedurende de nacht (5,6). Anti-inflamma-

toire (ontstekingsremmende) medicijnen zoals inhalatie corticosteroïden (ICS) onderdrukken de mate van inflammatie en beperken de circadiane variatie in de piekstroom (PEF) (7,8). Aangetoond is dat langwerkende luchtwegverwijdende $ß_2$-agonisten bij patiënten met astma met name effectief zijn om de nachtelijke daling in longfunktie te overbruggen (9). In de eerste studies met deze langwerkende $ß_2$-agonisten werd gesuggereerd dat deze medicijnen ook anti-inflammatoire eigenschappen zouden hebben, omdat een éénmalige inhalatie zowel de vroege als ook de late astmatische reactie na allergeen inhalatie zou voorkomen (10). Mede hierdoor ingegeven bestudeerden wij het effect, zowel overdag als 's nachts, van een 16 weken durende behandeling met de langwerkende $ß_2$-agonist salmeterol. We onderzochten het effect op de longfunktie, op de mate van bronchiale hyperreaktiviteit en op inflammatoire variabelen in het bloed van allergische kinderen met astma met een onderhoudsbehandeling van ICS.

Hoofdstuk 1.1 laat een literatuuroverzicht zien van de epidemiologie van nachtelijke klachten bij astma in verschillende groepen. Hierin worden onderliggende modulerende mechanismen besproken die het circadiane verloop van de longfunktie beïnvloeden. Het wordt algemeen geaccepteerd dat inflammatie het onderliggende pathologisch mechanisme is van astma. De circadiane variatie van het endogenene ritme zoals bronchiale hyperreaktiviteit, het autonome zenuwstelsel en de cortisol secretie moduleren het inflammatoire proces in de luchtwegen. Dit resulteert in een variabele luchtwegdiameter gedurende 24 uur. In ons model van nachtelijke luchtwegvernauwing bij kinderen met astma, veronderstellen we dat de ernst van het onderliggende inflammatoire proces verergerd wordt door uitlokkende omgevingsfactoren zoals expositie aan allergenen en niet-allergische prikkels, resulterend in een grotere 24 uurs variatie van de longfunktie.

In *Hoofdstuk 2* worden de resultaten besproken van een studie waarin we het voorkomen van nachtelijke klachten zoals hoesten, piepen, kortademigheid en ochtendbenauwdheid onderzochten. In deze studie bestudeerden we de nachtelijke klachten van 796 kinderen met astma die opeenvolgend onze polikliniek voor kinderlongziekten bezochten. Nachtelijke klachten werden door 47% van de kinderen gemeld; 6,3% had elke nacht klachten en 34% had tenminste 1x per week klachten passend bij nachtelijke luchtwegvernauwing. Slechts 38% van de 47% die nachtelijke klachten melden, meldde dit spontaan aan de arts. Patiënten met nachtelijke klachten hadden een lagere FEV_1, ervaren hun astma ernstiger en hun dagelijkse activiteiten werden meer beïnvloed door astma dan bij de groep kinderen zonder nachtelijke klachten van astma. Een éénmalig op de polikliniek geblazen FEV_1 blijkt een slechte voorspellende waarde te hebben voor nachtelijke klachten. Deze resultaten bevestigen dat nachtelijke klachten tengevolge van astma een ernstiger vorm van astma weerspiegelen. Bovendien moeten artsen specifiek naar nachtelijke klachten vragen omdat deze niet spontaan gemeld wordt door de

patiënt. Nachtelijke klachten bieden een opening om adequate therapie te introduceren.

In *Hoofdstuk 3* hebben we getracht de vraag te beantwoorden of het nivo van blootstelling aan huisstofmijt (HSM) in woon- en slaapkamers van 25 kinderen met astma hoger is dan bij gezonde kinderen in dezelfde leeftijd en van het zelfde geslacht, die in dezelfde omgeving wonen. Huisstofmijtallergeen (HSMA) concentraties waren niet significant verschillend tussen de 2 groepen, hoewel er een hogere schoonmaakfrequentie gevonden werd en er meer gladde vloerbedekking lag in de huizen van de groep kinderen met astma. We vonden dat bij deze Nederlandse schoolgaande kinderen in Nederland over het algemeen lagere HSMA concentratie dan in andere landen gemeld wordt. Gladde vloerbedekking bevatte significant minder stof en een lagere HSMA concentratie in vergelijking met vaste vloerbedekking. De grote inter-individuele verschillen in HSMA concentratie in de verschillende huizen rechtvaardigd het geven van individuele saneringsadviezen.

Hoofdstuk 4 beschrijft de resultaten van een studie waarbij we bij 55 kinderen met astma en een mono-allergie voor HSM de bijdrage van omgevingsfactoren, zoals het blootstellen aan sigarettenrook, de aanwezigheid van huisdieren en van de blootstelling aan HSMA in woon- en slaapkamers, in matrassen (n = 25) en in het schoollokaal, bestudeerden aan een toegenomen schommeling in piekstroom (PEF) amplitude (gedurende 24 uur elke 4 uur een PEF waarde blazen, amplitude berekend als hoogste min laagste waarde uitgedrukt als percentage van het daggemiddelde). Alle kinderen werden adequaat behandeld met dagelijks onderhoudsmedicatie bestaande uit ICS. Om de invloed van deze prikkels op de circadiane variatie in PEF amplitude te onderzoeken werden de ICS gedurende 6 dagen voor de metingen gestopt. We vonden dat het blootstellen aan sigarettenrook, de aanwezigheid van huisdieren en een hoge HSMA concentratie in het matras, ieder voor zich bijdroegen aan een toegenomen PEF amplitude. De hoogste HSMA concentratie werd gevonden in matrassen én op gladde vloeren waarop een los tapijt ligt.

In *Hoofdstuk 5* worden de resultaten gepresenteerd van een studie waarbij we onderzochten of het seizoensverschil in HSMA expositie bijdraagt aan een toename van de circadiane schommeling van de PEF amplitude bij 25 kinderen met astma die een mono-allergie voor HSM hebben. Bij alle kinderen werden d.m.v. het verzamelen van stofmonsters, de hoeveelheid HSMA bepaald in de woon- en slaapkamer en op het matras. In zowel de lente als in de herfst werden PEF amplitudes gemeten tijdens het gebruik van ICS en op de zesde dag na het staken van ICS. Deze cross-sectionele studie laat zien dat een hogere HSMA expositie in het matras niet geassocieerd is met een hogere PEF amplitude. De seizoensverandering in HSMA expositie (herfstwaarde minus lentewaarde), evenwel, draagt significant

bij aan de verandering in PEF amplitude na het staken van ICS.
De resultaten van de studies beschreven in hoofdstuk 4 en 5 ondersteunen de veronderstelling dat blootstelling aan exogene factoren, naast endogene factoren, de grootte van de circadiane variatie in de luchtwegdiameter beïnvloeden.

Hoofdstuk 6 is een *letter to the editor* als reactie op een studie van De Lovinfosse *et al.* gepubliceerd in *Allergy* 1994; 49: 64-66 waarin de auteurs een correlatie lieten zien tussen HSM specifiek IgE en de HSMA concentratie en suggereerden dat HSM specifiek IgE gebruikt kan worden als een alternatief voor HSMA expositie. Bij 25 kinderen met astma en een geïsoleerde allergie voor HSM hebben we getracht om dezelfde variabelen te correleren (serum HSM specifiek IgE en de HSMA concentratie op matrassen). Wij vonden geen correlatie tussen beide variabelen en konden de veronderstelling van De Lovinfosse *et al.* niet bevestigen. Dit lijkt ons inziens aannemelijk omdat de individuele immunologische mogelijkheid om te reageren op een bepaalde hoeveelheid HSM waarschijnlijk een meer relevante faktor is die de IgE produktie beïnvloedt.

In *Hoofdstuk 7* presenteren wij de resultaten van een 16 weken durende studie waar 40 kinderen met astma die al langere tijd behandeld werden met een ICS en voor deze studie *at random* behandeld werden met het langwerkende luchtwegverwijdende salmeterol of met placebo. De effecten op FEV_1 en op bronchiale hyperreaktiviteit, beide zowel overdag als 's nachts gemeten, werden onderzocht. Bovendien hebben we gekeken of het staken van salmeterol na 4 maanden gebruik, leide tot een toename in bronchiale hyperreaktiviteit als een 'rebound' effect. We vonden in de salmeterol groep een aanhoudend hogere FEV_1 en een verbetering in circadiane variatie van de luchtwegdiameter in vergelijking met de placebo groep gedurende 1 - 16 weken. De mate van bronchiale hyperreaktiviteit gedurende 1-16 weken was niet significant verschillend tussen beide groepen. Het achterwege blijven van verbetering van de bronchiale hyperreactiviteit kon niet verklaard worden door een 'plafond' effect omdat alle kinderen een matige tot ernstige bronchiale hyperreaktiviteit hadden en er voldoende ruimte voor verbetering mogelijk was. Staken van salmeterol na 16 weken dagelijks gebruik, gaf geen rebound verslechtering van de bronchiale hyperreaktiviteit.

In *Hoofdstuk 8* presenteren we een ander onderdeel van deze zelfde studie zoals beschreven in hoofdstuk 7. We onderzochten of toevoeging van salmeterol aan de behandeling met ICS leidt tot een verbetering van inflammatoire variabelen in bloed, zowel overdag als 's nachts. Behalve deze vraag hebben we gekeken of deze parameters bij allergische kinderen met astma, die dagelijks ICS gebruiken, verschillen van gezonde kinderen. We vonden dat kinderen met astma, ondanks behandeling met ICS, nog steeds een verhoogde activatie van eosinofiele cellen hebben, zowel overdag als 's nachts, en een toegenomen activatie van de lymfocy-

ten overdag. Eosinofiele activatie is gecorreleerd aan de variatie in luchtwegdiameter, terwijl de lymfocyten activatie geassocieerd is met de hoogte van de FEV_1. Ondanks klinische verbetering door de behandeling met salmeterol konden we geen verbetering van inflammatoire parameters overdag aantonen, terwijl er genoeg ruimte was voor verbetering gezien de waargenomen verschillen in inflammatoire parameters tussen de gezonde kinderen en de kinderen met astma.

Slotconclusies en aanbevelingen

Nachtelijke klachten komen veelvuldig voor in een populatie kinderen met astma die onder controle staan op een specialistische polikliniek voor astma. FEV_1 heeft een slecht voorspellende waarde voor nachtelijke klachten bij astma. Artsen moeten specifiek vragen naar nachtelijke symptomen omdat dit een ingang biedt om adequate behandeling te nemen. Na het afronden van deze epidemiologische studie kwamen er nieuwe therapeutische mogelijkheden zoals matrashoezen en medicijnen speciaal voor nachtelijke klachten. Het lijkt zeer de moeite waard om deze studie in de nabije toekomst te herhalen omdat verder onderzoek meer inzicht zal geven in een mogelijk veranderd patroon van nachtelijke klachten binnen deze populatie.

De studies in dit proefschrift geven nieuwe inzichten in de gedachtengang rond de pathofysiologie van nachtelijke luchtwegvernauwing bij kinderen met astma. Exogene factoren zoals blootstelling aan sigarettenrook, de aanwezigheid van huisdieren en een hoge blootstelling aan HSMA in het matras, dragen alle individueel bij aan de circadiane PEF amplitude van allergische astmatische kinderen. HSMA uit matrassen draagt het meeste bij aan de circadiane variatie in luchtwegdiameter in vergelijking met andere HSMA bronnen. Deze studie laat zien dat ouders niet alleen moeten stoppen met roken tijdens de zwangerschap, maar ook daarna niet meer moeten roken om de stabiliteit en de prognose van het astma van hun kind te verbeteren. Een andere belangrijke conclusie is dat huisdieren bijdragen aan een toename in circadiane PEF variatie, zelfs bij kinderen met astma die geen aantoonbare allergie hebben voor deze dieren. Het is aan te raden om een ander onderkomen voor huisdieren te zoeken om de stabiliteit van het astma te bevorderen.
Kinderen met astma worden blootgesteld aan vergelijkbare HSMA hoeveelheden als gezonde kinderen. Kinderen met astma hebben vaker gladde vloerbedekking in de woon- en slaapkamer, waarschijnlijk als een gevolg van eerder gegeven informatie omtrent het aanpassen van de woonomgeving. Gladde vloeren hebben een lagere HSM expositie als vaste vloerbedekking. Dit betekent dat bij het geven van adviezen aandacht besteed moet worden aan het verwijderen van vaste vloerbedekking.

Het concept dat gesuperponeerde endogene 24 uurs ritmes, zoals de bronchiale hyperreaktiviteit, van het autonome zenuwstelsel en van de cortisol secretie een belangrijke rol spelen bij de 24 uurs veranderingen van het ontstekingsproces in de

luchtwegen behoeft verdere onderbouwing.
Het is een aantrekkelijke hypothese om te veronderstellen dat de endogene cortisol secretie dit ontstekingsproces regisseert. Lage cortisolspiegels gedurende de nacht of een in het algemeen lagere cortisolsecretie bij kinderen met astma en nachtelijke symptomen versterken mogelijk het inflammatoire proces. Dit dient nader onderzocht te worden.

Langwerkende ß$_2$-adrenerge luchtwegverwijders zijn een goed alternatief in de behandeling van nachtelijke luchtwegbeperking. We vonden een aanhoudend luchtwegverwijdend effect en een vermindering van de 24 uurs variatie van de luchtwegdiameter bij kinderen met astma die naast hun ICS met salmeterol behandeld werden. We konden geen beschermend effect op de bronchiale hyperreaktiviteit aantonen, hoewel alle kinderen de mogelijkheid hadden om te verbeteren. We vonden geen rebound effect in bronchiale hyperreaktiviteit na het staken van salmeterol. Dit alles geeft weinig aanleiding om te veronderstellen dat salmeterol anti-inflammatoire eigenschappen heeft.
Nadere studies naar de behandeling van nachtelijke klachten van allergisch astmatische kinderen moeten gericht zijn op een optimaal therapeutisch regime. Matrashoezen en langwerkende luchtwegverwijders zoals salmeterol lijken succesvolle interventies, wanneer ze gecombineerd worden met anti-inflammatoire therapie zoals ICS. Desalniettemin zou stoppen met roken en het afschaffen van huisdieren ten zeerste geadviseerd moeten worden aan elk gezin met een kind met instabiel astma.

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Stellingen

behorend bij het proefschrift

Epidemiology and Exogenous Factors in Nocturnal Airflow Limitation in Children

29 mei 1996

Gerda Rosman-Meijer

I Nachtelijke luchtwegklachten bij kinderen zoals hoesten, piepen, kortademigheid en benauwdheid komen frequent voor, men moet er echter wel naar vragen.

II Gezinnen met kinderen met astma hebben vaker gladde vloerbedekking en daardoor een lagere huisstofmijtallergeen expositie dan gezinnen met gezonde kinderen.

III Blootstelling aan sigarettenrook, huisstofmijt en huisdieren draagt bij aan een toegenomen piekstroom amplitude bij kinderen met astma.

IV De seizoensschommeling in huisstofmijtallergeen expositie draagt bij aan een seizoensschommeling van het circadiane beloop van de longfunktie.

V Salmeterol verbetert de nachtelijke FEV_1 ook op lange termijn, doch niet de nachtelijke bronchiale hyperreaktiviteit.

VI Kinderen met astma gebruiken minder vaak hun dagelijks anti-inflammatoire medicatie dan is voorgeschreven. Luchtwegverwijders worden vaker dagelijks gebruikt dan is voorgeschreven.

VII Een donzen kussen is des duivels oorkussen.

VIII Een passief roker met astma is de sigaar.

IX Zoals hun ouders longen, piepen die van de jongen.

X Adviezen rond roken in relatie tot astma zijn maar al te vaak vrijblijvend.

XI Het routinematig aanvragen van een uitgebreide allergietest bij een zuigeling met luchtwegproblemen is niet zinvol.

XII *'t kon minder* en *goed te pas* geven dezelfde anamnestische informatie.